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(54)【発明の名称】 新遺伝子ペプチド

(57)【要約】

【課題】NF- $\kappa$ Bの活性化が関与する疾患の治療薬、予防薬および診断薬の探索、開発に有用なポリペプチド、該ポリペプチドをコードするDNA、該DNAのアンチセンスDNA/RNA、該DNAを用いた遺伝子治療、該ポリペプチドを認識する抗体、該ポリペプチドの活性上昇変体、該ポリペプチドのドミナントネガティブ変異体、およびこれらの利用法を提供する。

【解決手段】NF- $\kappa$ Bを活性化するポリペプチドを同一し、該ポリペプチドをコードするDNA、および該ポリペプチドを認識する抗体を製造する。これらはNF- $\kappa$ Bの活性化が関与する疾患の治療薬の探索ならびに診断に利用することができる。

【特許請求の範囲】

- 【請求項1】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列を有するポリペプチド。
- 【請求項2】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列において1以上のアミノ酸が欠失、置換および/または付加されたアミノ酸配列からなり、かつNF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチド。
- 【請求項3】 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列と60%以上の相同性を有するアミノ酸配列を含み、かつNF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチド。
- 【請求項4】 請求項1～3のいずれか1項に記載のポリペプチドをコードするDNA。
- 【請求項5】 配列番号6～10のいずれかで表される塩基配列を有するDNA。
- 【請求項6】 請求項4または5に記載のDNAとストリンジェントな条件下でハイブリダイズするDNAであり、かつ転写因子NF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチドをコードするDNA。
- 【請求項7】 請求項4～6のいずれか1項に記載のDNAをベクターに組み込んで得られる組換え体ベクター。
- 【請求項8】 請求項4～6のいずれか1項に記載のDNAと相同な配列からなるRNAをベクターに組み込んで得られる組換え体ベクター。
- 【請求項9】 RNAが1本鎖である請求項8記載の組換え体ベクター。
- 【請求項10】 請求項7記載の組換え体ベクターを保有する形質転換体。
- 【請求項11】 形質転換体が、微生物、動物細胞、植物細胞、および昆虫細胞からなる群より選ばれた形質転換体である、請求項10記載の形質転換体。
- 【請求項12】 微生物が、Escherichia属に属する微生物である、請求項11記載の形質転換体。
- 【請求項13】 動物細胞が、マウス・ミエローマ細胞、ラット・ミエローマ細胞、マウス・ハイブリドマ細胞、CHO細胞、BHK細胞、アフリカミドリザル腎臓細胞、Natalwa細胞、Natalwa KJM細胞、ヒト胎児腎臓細胞およびヒト白血病細胞から選ばれた動物細胞である、請求項11記載の形質転換体。
- 【請求項14】 昆虫細胞が、Spodoptera frugiperdaの卵巣細胞、Tetrahymenaの卵巣細胞およびカイロの卵巣細胞から選ばれた昆虫細胞である、請求項11記載の形質転換体。
- 【請求項15】 形質転換体が、非ヒトトランスジェニック動物またはトランスジェニック植物である、請求項11記載の形質転換体。

10記載の形質転換体。

- 【請求項16】 請求項10～14のいずれか1項に記載の形質転換体を宿主に感染し、培養物中に請求項1～3のいずれか1項に記載のポリペプチドを生成、蓄積させ、該培養物から該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。
- 【請求項17】 請求項7記載の組換え体DNAを保有する非ヒトトランスジェニック動物を飼育し、請求項1～3のいずれか1項に記載のポリペプチドを該動物中に生成、蓄積させ、該動物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。
- 【請求項18】 畜産が動物のミルク中であることを特徴とする、請求項17記載の製造法。
- 【請求項19】 請求項7記載の組換え体DNAを保有するトランスジェニック植物を栽培し、請求項1～3のいずれか1項に記載のポリペプチドを該植物中に生成、蓄積させ、該植物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造法。
- 【請求項20】 請求項4～6のいずれか1項に記載のDNAを用い、in vitroでの転写・翻訳系により、該DNAのコードするポリペプチドを合成することを特徴とする、該ポリペプチドの製造法。
- 【請求項21】 請求項1～3のいずれか1項に記載のポリペプチドを認識する抗体。
- 【請求項22】 請求項4～6のいずれか1項に記載のDNAの塩基配列中の連続した5～60塩基からなる配列を有するオリゴヌクレオチドまたはヌクレオチドと相補的な配列を有するオリゴヌクレオチド。
- 【請求項23】 請求項4～6のいずれか1項に記載のDNAまたは請求項22記載のオリゴヌクレオチドをプローブとして用いてハイブリダイゼーションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの発現を検出する方法。
- 【請求項24】 請求項22記載のオリゴヌクレオチドをプライマーとして用いたポリメラーゼ・チェイン・リアクションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの発現を検出する方法。
- 【請求項25】 請求項4～6のいずれか1項に記載のDNAまたは請求項22記載のオリゴヌクレオチドを用い、ハイブリダイゼーション法により、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの変異を検出する方法。
- 【請求項26】 請求項22記載のオリゴヌクレオチドを用いたポリメラーゼ・チェイン・リアクションを行うことを含む、請求項1～3のいずれか1項に記載のポリペプチドをコードするDNAの変異を検出する方法。
- 【請求項27】 感染や炎症に伴う疾患、異常な平滑筋細胞の分化増殖に伴う疾患、異常な繊維芽細胞の活性化に伴う疾患、異常な増殖組織の活性化に伴う疾患、腫瘍





【0015】さらに、エイズ等、癌以外のNF- $\kappa$ Bを転写因子として含むウイルス性疾患においても、NF- $\kappa$ Bは重要な創薬あるいは治療ターゲットである。また、虚血性脳虚損等の虚血再灌流障害もNF- $\kappa$ B活性化による細胞障害、アポトーシスの抑制等が原因という報告があり、動脈硬化、再狭窄等も含め、平滑筋細胞の異常な分化増殖を伴う疾患の発症にNF- $\kappa$ Bが重要な役割を果たしていると考えられる。

【0016】近年ステロイドの抗炎症作用やアスピリンの抗炎症作用等がNF- $\kappa$ Bの阻害によるものであることが明らかにされたが、Nakanishi *et al.* (1995), Science, 270, 286-290 (1995), Molecular and Cellular Biology, 15, 943-953 (1995)), NF- $\kappa$ Bを特異的に阻害するものとしてスクレニンニグされた薬剤はない。既存のNF- $\kappa$ Bの阻害に関わるものとして知られてきた薬剤は副作用が強いことや選択性、特異性が低い等、問題点も多く、強力かつ副作用の少ない新しい抗炎症薬の開発を目的として、NF- $\kappa$ Bをターゲットにした化合物探索が行われている。以上より、NF- $\kappa$ Bを活性化させる新規ポリペプチドは産業上有用であり、これらポリペプチドおよびそれをコードするDNAの取得が求められてきた。

【0017】**【発明が解決しようとする課題】**本発明は、アレキサンダー、アトピー、喘息、花粉症、自己免疫疾患、移植片対宿主型免疫反応等の異常な免疫増殖を伴う疾患、エンドキニンシク、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性非依存性糖尿病、糸球体腎炎、乾眼、痛風、各種脳神経炎、うつ血性心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、パーキンソン病、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎、エイズ等のウイルス性疾患、虚血性脳虚損の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化、再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群(SIRS: systemic inflammatory response syndrome)、成人呼吸器障害症候群(ARDS: adult respiratory distress syndrome)等の治療薬、予防薬および診断薬の探索、開発に有用なポリペプチド、該ポリペプチドをコードするDNA、該DNAのアンチセンスDNA/RNA、該DNAを用いた遺伝子治療、該ポリペプチドを認識する抗体、該ポリペプチドの活性化上昇変異体、該ポリペプチドのドミナントネガティブ変異体、およびこれらの利用法を提供することにある。

【0018】

【課題を解決するための手段】本発明者らは、上記課題を

を解決するべく鋭意検討を行った結果、新規なアミノ酸配列を含むNF- $\kappa$ Bの活性化を促す因子および該因子をコードするDNAを取得することに成功し、本発明を完成させるに至った。即ち、本発明は以下の(1)～(54)に関する。

【0019】(1) 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列を有するポリペプチド。

(2) 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列において1以上のアミノ酸が欠失、置換および/または付加されたアミノ酸配列からなり、かつNF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチド。

【0020】(3) 配列番号1～5のいずれかで表されるアミノ酸配列からなる群より選ばれたアミノ酸配列を含む、かつ60%以上の相同性を有するアミノ酸配列を含む、かつNF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチド。

(4) (1)～(3)のいずれか一項に記載のポリペプチドをコードするDNA。

(5) 配列番号6～100のいずれかで表される塩基配列を有するDNA。

【0021】(6) (4)または(5)に記載のDNAとストリンジェントな条件下でハイブリダイズするDNAであり、かつ転写因子NF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチドをコードするDNA。

(7) (4)～(6)のいずれか一項に記載のDNAをベクターに組み込んで得られる組換え体ベクター。

(8) (4)～(6)のいずれか一項に記載のDNAと相同な配列からなるRNAをベクターに組み込んで得られる組換え体ベクター。

【0022】(9) RNAが1本鎖である(8)に記載の組換え体ベクター。

(10) (7)に記載の組換え体ベクターを保有する形質転換体。

(11) 形質転換体が、微生物、動物細胞、植物細胞、および昆虫細胞からなる群より選ばれた形質転換体である、(10)に記載の形質転換体。

(12) 微生物が、Escherichia属に属する微生物である、(11)に記載の形質転換体。

【0023】(13) 動物細胞が、マウス・ミエローマ細胞、ラット・ミエローマ細胞、マウス・ハイブリドーマ細胞、CHO細胞、BHK細胞、アフリカミドリザル腎臓細胞、Namalwa細胞、Namalwa K JM-1細胞、ヒト胎児腎臓細胞およびヒト白血病細胞から選ばれた動物細胞である、(11)に記載の形質転換体。

(14) 昆虫細胞が、Spodoptera frugiperdaの卵巣細胞、Trichoplusia niの卵巣細胞およびカイコの卵巣細胞から選ばれた昆虫細胞である、(11)に記載の形質

転換体。

【0024】(15) 形質転換体が、非ヒトトランスジェニック動物またはトランスジェニック植物である、(10)に記載の形質転換体。

(16) (10)～(14)のいずれか一項に記載の形質転換体を細胞に接種し、培養物中に(1)～(3)のいずれか一項に記載のポリペプチドを生成、蓄積させ、該培養物から該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。

【0025】(17) (7)に記載の組換え体DNAを保有する非ヒトトランスジェニック動物を飼育し、(1)～(3)のいずれか一項に記載のポリペプチドを該動物中に生成、蓄積させ、該動物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造方法。

(18) 畜種が動物のミルグ中であることを特徴とする、(17)に記載の製造法。

【0026】(19) (7)に記載の組換え体DNAを保有するトランスジェニック植物を栽培し、(1)～(3)のいずれか一項に記載のポリペプチドを該植物中に生成、蓄積させ、該植物中より該ポリペプチドを採取することを特徴とする、該ポリペプチドの製造法。

(20) (4)～(6)のいずれか一項に記載のDNAを用い、in vitroでの転写・翻訳系により、該DNAをコードするポリペプチドを合成することを特徴とする、該ポリペプチドの製造法。

【0027】(21) (1)～(3)のいずれか一項に記載のポリペプチドを認識する抗体。

(22) (4)～(6)のいずれか一項に記載のDNAの塩基配列中の連続した5～6塩基からなる配列と有するオリゴヌクレオチドまたは該オリゴヌクレオチドと相補的な配列を有するオリゴヌクレオチド。

(23) (4)～(6)のいずれか一項に記載のDNAまたは(22)に記載のオリゴヌクレオチドをプローブとして用いてハイブリダイゼーションを行うことを含む、(1)～(3)のいずれか一項に記載のポリペプチドをコードするDNAの発現を検出する方法。

(24) (22)に記載のオリゴヌクレオチドを含む、(1)～(3)のいずれか一項に記載のポリペプチドをコードするDNAの発現を検出する方法。

(25) (4)～(6)のいずれか一項に記載のDNAまたは(22)に記載のオリゴヌクレオチドを用い、ハイブリダイゼーションにより、(1)～(3)のいずれか一項に記載のポリペプチドをコードするDNAの変異を検出する方法。

【0029】(26) (22)に記載のオリゴヌクレオチドを用い、ポリメラゼ・チェーン・リアクションを行うことを含む、(1)～(3)のいずれか一項に記載

のポリペプチドをコードするDNAの変異を検出する方法。

(27) 感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性化を伴う疾患、異常な滑膜組織の活性化を伴う疾患、臓癌細胞の増殖を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、または異常な細胞増殖を伴う疾患を検出するために用いる、(23)～(26)のいずれか一項に記載の方法。

【0030】(28) 感染や炎症を伴う疾患が、微生物感染、HIV感染、慢性B型肝炎に代表される活動性慢性肝炎、慢性関節リウマチ、糸球体腎炎、乾眼、痛風、各種脳神経炎、うつ血性心不全、エンドキニンシク、敗血症、移植片対宿主型疾患、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異常な線維芽細胞の活性化を伴う疾患が肺線維症であり、異常な滑膜組織の活性化を伴う疾患がリウマチ性関節炎または変形性関節炎であり、臓癌細胞の増殖を伴う疾患が腫瘍であり、異常な破骨細胞の活性化を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、気道過敏症または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または悪性腫瘍である、(27)に記載の方法。

【0031】(29) (4)～(6)のいずれか一項に記載のDNAまたは(22)に記載のオリゴヌクレオチドを用いることを特徴とする、(1)～(3)のいずれか一項に記載のポリペプチドをコードするDNAの転写またはmRNAの翻訳を抑制する方法。

(30) (4)～(6)のいずれか一項に記載のDNAまたは(22)に記載のオリゴヌクレオチドを用いることを特徴とする、(1)～(3)のいずれか一項に記載のポリペプチドをコードするDNAのプロモーター領域および転写制御領域を取得する方法。

【0032】(31) (1)～(3)のいずれか一項に記載のポリペプチドを含む、医薬。

(32) (4)～(6)のいずれか一項に記載のDNA、または(8)若しくは(9)のいずれか一項に記載の組換え体ベクターを含む医薬。

(33) (21)に記載の抗体を含む医薬。

(34) (22)に記載のオリゴヌクレオチドを含む医薬。

【0033】(35) ポリペプチドが免疫賦活作用を有することを特徴とする(31)に記載の医薬。

(36) 免疫賦活作用を介して抗腫瘍活性および抗ウイルス活性を誘導することを特徴とする(35)に記載の医薬。

(37) 医薬が、感染や炎症を伴う疾患、異常な平滑筋細胞の分化増殖を伴う疾患、異常な線維芽細胞の活性

化を伴う疾患、異常な清血組織の活性化を伴う疾患、脾臓β細胞の障害を伴う疾患、異常な破骨細胞の活性化を伴う疾患、異常な免疫細胞の活性化を伴う疾患、異常な細胞増殖を伴う疾患または神経細胞の障害に基づく疾患の治療および／または予防のための医薬である。(32)〜(34)のいずれか1項に記載の医薬。

【0034】(38) 癌系が、感染や炎症を伴う疾患、癌系が平滑筋細胞の分化・増殖を伴う疾患、癌系が線維芽細胞の活性化を伴う疾患、癌系が平滑筋組織の活性化を伴う疾患、腫瘍細胞の増殖を伴う疾患、癌系が骨形成細胞の活性化を伴う疾患、癌系が免疫細胞の活性化を伴う疾患、または癌系が細胞増殖を伴う疾患の診断のための癌系である、(32)～(34)のいずれか1項に記載。

【0035】(39) 感染性炎症を伴う疾患が、發生物感症、HIV感染症、慢性肝炎に代表される活動性慢性肝炎、慢性腎臓病リウマチ、糸球体腎炎、乾癆、痛風、各種虚性熱、うつ血性不全、エントカシンシンドローム、各種虚性熱、うつ血性不全、インスリン依存性糖尿病、外傷性脳損傷または炎症性腸疾患であり、異常な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または骨髄造血組織の活性化に伴う疾患が肺がん、肺癌であり、異常な樹状芽細胞の活性化を伴う疾患が師乳癌であり、異常な骨髄造血細胞の活性化を伴う疾患が淋巴瘤性白血病であり、異常な骨髄造血細胞の活性化を伴う疾患が急性骨髄性白血病であり、脾臓β細胞の活性の障害を伴う疾患が糖尿病であり、異常な破骨細胞の活性を伴う疾患が骨粗鬆症であり、異常な免疫細胞の活性化を伴う疾患がアレルギー、アトピー、喘息、花粉症、氣道過敏または自己免疫疾患であり、異常な細胞増殖を伴う疾患が急性骨髄性白血病または慢性骨髄性白血病、神経細胞の障害に基づいた疾患がアルツハイマー病または虚血性脳疾患である。(37) または (38) 記載の要素。

【0036】(40) (1)～(3)のいずれか1項に記載のポリペプチドを用いることを特徴とする、感染や炎症を伴う免疫、異種な線維芽細胞の分化増殖を伴う疾患、異種な破骨細胞の活性化を伴う疾患、異種な癌組織の活性化を伴う疾患、脾臓が細菌障害を伴う疾患、異種な破骨細胞の活性化を伴う疾患、異種な免疫細胞の活性化を伴う疾患、異種な細胞増殖を伴う疾患またはその治療法。また、本発明は、上記の任意の組み合わせによるものを含む。

【0037】(41) 感染が炎症を伴う疾患が、微生物感染、HIV感染、慢性肝面筋炎に代表される活動性慢性肝炎、肥厚性硬縮肝マウス、糸状体腎臓、乾癆、痛風、各種慢性骨髄炎、うつ病による心不全、インドロンキンゼンヨック、敗血症、移植した血行性疾患、インスリン依存性糖尿病内、外傷性脳損傷または炎症性腸炎疾患であり、異質な平滑筋細胞の分化増殖を伴う疾患が動脈硬化または再狭窄であり、異質な線維芽細胞の活性化に伴う疾患が肺気腫であり、異質な平滑筋組織の活性化を伴う疾患が閉塞性血管疾患であり、異質な平滑筋細胞の増殖を伴う疾患が

(47) (21) 記載の抗体を用いて、(1)～(3)のいずれか1項に記載のポリペプチドを検出することを特徴とする免疫組織染色法。

【0041】(48) 記載の抗体を用いることを特徴とする、(1)～(3)のいずれか一項に記載のポリヌクレオチドをコードするDNAの転写もしくは翻訳を抑制または促進する物質をスクリーニングする方法。

(49) (1)~(3)のいずれか1項に記載のポリペプチドをコードするDNAの発現が一部または完全に抑制されているノックアウト非ヒト動物。

(50) (1)～(3)のいずれか1項に記載のポリペプチドの有する活性が一部または完全に抑制されているノックアウト非ト動物。

【0042】(51) (1)～(3)のいずれか1項に記載のポリペプチドを用いることを特徴とする、  
(1)～(3)のいずれか1項に記載のポリペプチドのNMF- $\kappa$ B活性化に対してドミナントネガティブ活性を有する変異体ポリペプチドのスクリーニング方法

(52) (51) 記載のスクリーニング方法により取得される、(1)～(3)のいずれか1項に記載のポリペプチドのNF- $\kappa$ B活性化に対してドミナントなゲナティブ活性を有する変異体ポリペプチド。

(53) 記載の変異体ポリペプチドをコードするDNA。

【0043】(54) (1)～(3)のいずれか1項に記載のポリペプチドを用いることを特徴とする、

(1) ~ (3) のいずれか1項に記載のポリペプチドの N F - x B 活性化に対して耐活性性を上昇させる変異を有する変異体ポリペプチドのスクリーニング方法。

(5) (4) 記載のスクリーニング方法により取得される、(1) ~ (3) のいずれか1項に記載のポリペプチドの N F - x B 活性化能が上昇した変異体ポリペプチド。

(56) (55) 記載の変異体ポリペプチドをコードするDNA。  
【0044】

【発明の実施の形態】本発明のポリペプチドとしては、  
 1. 配列番号1～5のいずれかで表されるアミノ酸配列  
 からなる群より選ばれるアミノ酸配列を有するポリペプ  
 チド

2. 配列番号 1 によって表されるアミノ酸配列からなる群  
より選ばれるアミノ酸配列において 1 以上のアミノ酸残基が、かつ N- $\alpha$  B の活性を有する  
アミノ酸、側鎖および/または付加されたアミノ酸配列からなる群より選ばれるアミノ酸配列を有する  
ポリペプチド、かつ N- $\alpha$  B の活性を有する

3. 配列番号 1 によって表されるアミノ酸配列からなる群より選ばれるアミノ酸配列と 60% 以上の相  
同性を有するアミノ酸配列を含む、かつ N- $\alpha$  B の活  
性を有するアミノ酸配列を含む、かつ N- $\alpha$  B の活  
性を有するポリペプチドを有するポリペプチドを有すること  
ができる。

【0045】上記のアミノ酸配列を有するポリペプチドにおいて、1以上のアミノ酸が缺失、置換および/または、加えられたアミノ酸配列を有するポリペプチドは、Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989 (以下、モレキュラー・クロニング第2版と略す)、Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1987 (以下、カレント・プロトコールズ・イン・モレキュラー・バイオロジーと略す)、Nucleic Acids Research, 10, 6487, (1982)、Proc. Natl. Acad. Sci., USA, 79, 6409 (1982)、Gene, 34, 315 (1985)、Nucleic Acids Research, 13, 4431 (1985)、Proc. Natl. Acad. Sci. USA, 82, 488 (1985)等に記載の部分特異的変異法を用いて、例は配列番号1〜50のいずれかのアミノ酸配列を有するポリペプチドにコードするDNAに部分特異的変異を導入することにより行うことができる。欠失、置換および/または付加されるアミノ酸の数は1から数個であり、その数は特知の技術により、欠失、置換もしくは付加する個所に限定されないが、上記の部分特異的変異導入法の周知の技術であり、例えば、1〜数十個、好ましくは1〜20個、より好ましくは1〜10個、さらに好ましくは1〜5個である。

【0046】また、本発明のポリペプチドとしては、配列番号1〜5のいずれかに記載のアミノ酸配列を60%以上の相同性を有するアミノ酸配列を含む、配列番号1〜5のいずれかに記載のアミノ酸配列との相違は、B L A S T (J. Mol. Biol., 215, 403 (1990)) や F A S T A (Methods in Enzymology, 183, 63-69)等の解析ソフトで、デフォルト(初め設定)のパラメータを用いて計算したときに、少なくとも60%以上、好ましくは70%以上、より好ましくは80%以上、さらに好ましくは90%以上、特に好ましくは95%以上、最も好ましくは97%以上と好ましい。

【0047】本発明のDNAとしては、

1. 請求項1～3のいずれか一項に記載のポリペプチドをコードするDNA
2. 請求項4記載のDNAとストリンジェントな条件下でハイブリダイズするDNAであり、かつ転写因子NF- $\kappa$ Bの活性を上昇させる活性を有するポリペプチドをコードするDNA

3. 配列番号6~10のいずれかで表される塩基配列を有するDNAを基げることができる。

【0048】一般に1つのアミノ酸に対して複数の遺伝的変換番号が存在するため、配列番号6-10のいずれかと変換番号を有するDNAであっても本発明のポリペプチドをコードしているかは不明である。したがって、本発明のポリペプチドをコードしているDNAに含められたいは、ストロンジェントな条件下でアブリダイズするDNAと見なされ、例えば配列番号6、7、8、9または10で示されるDNA等の本発明のDNAと見なされる。本発明のDNA等には、本発明のポリペプチドをコードしているDNAに含められたいは、ストロンジェントな条件下でアブリダイズするDNAと見なされ、例えば配列番号6、7、8、9または10で示されるDNAと見なされる。



ama Iwa (ATCC CRL-1432)、子宮癌細胞系 Hela (ATCC CCL-2)、腎細胞株 293 U. Gen. Viol. 3 (6, 59-72 (1977)) 等を用いることができる。ヒト以外の哺乳動物の細胞としては、サル腎細胞株 COS-7 (ATCC CRL-16 50)、サル腎細胞株 COS-7 (ATCC CRL-1651)、チャイニーズ・ハムスター卵巣 (Chinese Hamster Ovary) 細胞株 CHO (ATCC CRL-9096、ATCC CRL-61)、マウス細胞株 Ba/F3 (RIKEN Cell Bank RC C88085)、マウス細胞株 L929 (RIKEN Cell Bank RC 08081)、ラット細胞株 FHK-49 F (ATCC CRL-1571)、ミンク細胞株 Mv1Lu (ATCC CRL-64) 等を用い

【0069】具体的には、IL-1 $\alpha$ 、IL-1 $\beta$ 、IL-2、IL-3、IL-6、IL-8、IL-12、TNF- $\alpha$ 、TNF- $\beta$ 、IFN- $\beta$ 、M-CSF、G-CSF、TGF- $\beta$ 、IL-2R $\alpha$ 、Ig- $\kappa$ -L C、T細胞リグンダー $\beta$ 、細胞クラス1、 $\beta$ 2-ミクログロブリン、LAM-1、VCAM-1、ICAM-1、血清アミロイド前駆タンパク質、アンギオテンシノーゲン、精細胞因子B、精細胞因子C、精細胞因子D、INO S、COX-2、VEGF-R2、c-ER $\beta$ 、p105、IkB $\alpha$ 、c-Myc、IKF-1、HIV-1、HIV-2、SVI $\alpha$ 、CMV、HSV-1、SV40、アデノウイルス等のプロモーターやそれらのコンセンサス配列に1個あるいは複数個有した合成プロモーター等が選択される。

バイオマニキュアルシリーズ4、28)、マイクロロビンジ  
ェクション法(幸社社 バイオマニキュアルシリーズ4、3  
6)、アデノウイルス法(幸社社 バイオマニキュアルシリ  
ーズ4、43)、ワクシニアウイルス法(幸社社 バイオ  
マニキュアルシリーズ4、59) レトロウイルスベクター法  
(幸社社 バイオマニキュアルシリーズ4、74) 等の公知  
の方法を用いることができる。

【0067】(3) 本発明のDNAを転導する方法  
本発明のDNAは、細胞で発現させることによりNF- $\kappa$ Bを活性化させるため、細胞におけるNF- $\kappa$ Bの活  
性を発出することが可能な方法を用いることにより、  
細胞のDNAを転導することができる。NF- $\kappa$ Bの活

【0071】3. 本発明のポリプレプチドの製造  
本発明のポリプレプチドは、モレキュラー・クロマトゲ  
第1段階とカレント・プロトコル・イン・モレキュラ  
ー・バイオロジー等に記載された方法等を用い、例えば  
以下に製造される。本発明のDNAを宿主細胞中で発現  
させて、製造することができる。

【0072】全長cDNAをもとにして、必要に応じて、該ポリペプチドをコードする部分を含む適當な長さのcDNA断片を調製する。該DNA断片、または全長cDNA断片を該当発現ベクターのプロモーターの下流に挿入することにより、組織とベクターを製作する。該組織発現ベクターを、該発現ベクターに適合した宿主細胞に導入することにより、本発明のポリペプチドを生産する形

質転換体と名づけることができる。

【0073】宿主細胞としては、細菌、酵母、動物細胞、昆虫細胞、植物細胞等、目的とする遺伝子を発現させるものを用いることができる。発現させるものとしては、上記宿主細胞において自発複製可能なクローンとしては、上記宿主細胞への組み込みが可能で、本発明のポリペプチドは該宿主中への組み込みが可能で、本発明のポリペプチドを転写するDNAを転写できる位置にプロモーターを含有するものを用いられる。

【0074】細菌等の原核生物を宿主細胞として用いる場合は、本発明のポリペプチドをコードするDNAを含む、あるいは本発明のポリペプチドをコードするDNAを含有してなる核酸ベクターは、原核生物中で自然発現可能であると同時に、プロモーター、リボソーム結合配列、本発明のポリペプチドをコードする遺伝子、および転写終止配列により構成されたベクターであることが好ましい。尚、ベクターには、プロモーターを制御する遺伝子が含まれることもよい。

【0073】発現ベクターとしては、例えば、pTrp2 (Boehringer Mannheim社製)、pBlacl (Boehringer Mannheim社製)、pBlaC2 (Boehringer Mannheim社製)、pK233-2 (Pharmacia社製)、pS280 (Invitrogen社製)、pCEEX-1 (Promega社製)、pGE-8 (GIBCO社製)、pVP10 (特開昭8-11060号)、pVP200 (Agricultural Biological Chemistry, 48, 669 (1989))、pLAI (Agric. Biol. Chem. 53, 277 (1989))、pGELI (Proc. Natl. Acad. Sci. USA, 82, 4306 (1985))、pGELI II SK(-) (Stratagene社製)。

(FERM) pTrcS30 [*Escherichia coli* JM109/pTrcS30 (FERM) 型], pTrcS32 [*Escherichia coli* JM109/pTrcS32 (FERM BP-5407) より構築], pTrcS33 [*Escherichia coli* JM109/pTrcS33 (FERM BP-5408) より構築], pGMA2 (特許第660-979号/pTrcS32 (FERM BP-5408) より構築), pGMA2A (特許第660-222091号)/pGMA2 [*Escherichia coli* ICAK2 (FERM BP-400) より構築, 特許第660-222091号)], pGMA2B (特許第660-222091号)/pGMA2 [*Escherichia coli* ICAK2 (FERM BP-400) より構築, 特許第660-222091号)], pGMA2C (米国特許第4,686,191号、米国特許第4,939,094号、および米国特許第4,965,341号、米系特許第1,160,735号)、pDxpec、pHLH10、pTFS、pC19 (米国特許第4,965,341号、米系特許第1,160,735号)、pDxpec、pHLH10、pTFS、pC19 (Pharmacia社) 等 EX (Pharmacia社) のpTFSシステム (Novogen社) を用いて構築することによって、リポソーム結合開始点であるシャイン・ダルガーノ (Shine-Dalgarno) 配列と開始コドンとの間を適当な距離(例えば、15個以上の塩基)を用いることができる。

[illegible]

【0077】本発明のポリヌクレオチドをコードする部分の塩基配列を、宿主の発現に適切なコドンとなるように最適化することにより、目的とするポリヌクレオチドの生産率向上させることができる。本発明の組織換えベクターにおいては、本発明のDNAの発現に転写後修飾（糖基化）が必要と必要でないが、糖基化伝達子の下に転写されるべき遺伝子に糖基化を配する必要がある。糖基化伝達子の配列は必ずしも必要でないが、糖基化伝達子の下に転写されるべき遺伝子に糖基化を配する必要がある。糖基化伝達子の配列は必ずしも必要でないが、糖基化伝達子の下に転写されるべき遺伝子に糖基化を配する必要がある。

[illegible]

【079】組換えベクターの導入方法としては、上記の宿主細胞にDNAを導入する方法であればいずれも用いることができる。例えば、カリウムイオンを用いる方法(Proc. Natl. Acad. Sci. USA, 69, 2110 (1972))、プロトプラスト法(特開昭63-248394号)、または、Gene. 17, 107 (1982) やMolecular & General Genetics, 168, 111 (1979) に記載の方法等を用いることができる。

【0080】酵母を宿主細胞として用いる場合には、発現ベクターとして、例えば、YEP13 (ATCC37115)、YEp2.0 (ATCC37051)、YEp50 (ATCC37419)、pJ5.9、pJ5.10等を用いることができる。プロモーターとしては、酵母由来の株中で発現できるものを用い、いずれのものも、例えば、ヘキサノキナーゼ等の解糖系の遺伝子プロモーター、P<sub>ADH1</sub>プロモーター、P<sub>ADH2</sub>プロモーター、P<sub>ADH3</sub>プロモーター、P<sub>ADH4</sub>プロモーター、P<sub>ADH5</sub>プロモーター、P<sub>ADH6</sub>プロモーター、P<sub>ADH7</sub>プロモーター、P<sub>ADH8</sub>プロモーター、P<sub>ADH9</sub>プロモーター、P<sub>ADH10</sub>プロモーター、ヒートショックタンパク質プロモーター、HPIプロモーター、CUP1プロモーター等を用いることができる。

【0081】 宿主細胞としては、サツカロミセス属、クリューベロミセス属、トリコスポロン属、シュワニオミセス属等に属する微生物、例えば、*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces fragilis*, *Trichosporon pullulans*, *Schwaaniomyces allii* 等。



【0087】バキュロウイルスとしては、例えば、夜盗蛾科昆虫に感染するウイルスであるアウトグラファ・カリフォルニカ・ヌクレアー・ポリヘドロシス・ウイルス (Autographa californica nuclear polyhedrosis virus) を用いることができる。昆虫細胞としては、*Spodoptera frugiperda* の卵巣細胞である Sf9、Sf21 [Baculovirus Expression Vectors, A Laboratory Manual, W. H. Freeman and Company, New York (1992)]、*Epiloplusia ni* の卵巣細胞である High5 (Invitrogen社製) 等を用いることができる。

【0088】組換えウイルスを調製するための、昆虫細胞への上記組換え遺伝子導入ベクターと上記バキュロウイルスの共導入方法としては、例えば、リン酸カルシウム法 (特開平2-227075)、リポフェクション法 [Proc. Natl. Acad. Sci. USA, 84, 7413 (1987)] 等を挙げることができる。植物細胞を宿主細胞として用いる場合は、発現ベクターとして、例えば、Tiプラスミド、タバコモザイクウイルスベクター等を挙げることができる。

【0089】プロモーターとしては、植物細胞中で発現できるものであればいずれのものを用いてもよく、例えば、カリフラワーモザイクウイルス (CaMV) の35Sプロモーター、イネアチナ1プロモーター等を挙げることができる。宿主細胞としては、タバコ、ジャガイモ、トマト、ニンジン、ダイズ、アブラナ、アルファルファ、イネ、コムギ、オオムギ等の植物細胞等を挙げることができる。

【0090】組換えベクターの導入方法としては、植物細胞にDNAを導入する方法であればいずれも用いることができ、例えば、アグロバクテリウム (*Agrobacterium*) (特開昭59-140885、特開昭60-70080、W094/00977)、エレクトロポレーション法 (特開昭60-251887)、ハーティクルガン (遺伝子銃) を用いる方法 (特許第260835、特許第2517813) 等を挙げることができる。

【0091】遺伝子の発現方法としては、直接発現以外に、モレキュラー・クロニング第2版に記載されている方法等に応じて、分泌生産、融合ポリペプチド発現等を行うことができる。酵母、動物細胞、昆虫細胞または植物細胞により発現させた場合には、糖あるいは糖鎖が付加されたポリペプチドを得ることができる。

【0092】本発明のDNAを組み込んだ組換え発現ベクターを保有する形質転換体を宿主細胞に培養し、培養物に本発明のポリペプチドを生産蓄積させ、該培養物より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。大腸菌等の原核生物あるいは酵母等の真核生物を宿主として得られた形質転換体を培養する宿主としては、該生物が質的に得る炭素源、窒素源、無機塩類等を含有し、形質転換体の培養を効率的に行える宿主であれば天然宿主、合成宿主のいずれを用いてもよい。

ウイルス等を挙げることができる。組換えベクターの導入方法としては、酵母にDNAを導入する方法であればいずれも用いることができ、例えば、エレクトロポーション法 (Methods, Enzymol., 194, 182 (1990))、スプレッドラスト法 [Proc. Natl. Acad. Sci. USA, 84, 1929 (1978)]、酢酸リチウム法 [J. Bacteriology, 153, 163 (1983)]、[Proc. Natl. Acad. Sci. USA, 75, 1929 (1978)] 記載の方法等を挙げることができる。

【0082】動物細胞を宿主として用いる場合には、発現ベクターとして、例えば、pCDM1、pCDM8 (フナコ社製)、pAGE107 (特開平3-22879; Cytotechnology, 3, 133 (1990))、pAS3-3 (特開平2-227075)、pCDM8 (Natu re, 329, 840 (1987))、pCDNA1/Amp (Invitrogen社製)、pRPA (Invitrogen社製)、pAGE103 [J. Biochem. Isty., 101, 1307 (1987)]、pAGE210等を挙げることができる。

【0083】プロモーターとしては、動物細胞中で発現できるものであればいずれも用いることができ、例えば、サイトメガロウイルス (CMV) のIE (immediate early) 遺伝子のプロモーター、SV40の初期プロモーター、レトロウイルスのプロモーター、メタロチオネインプロモーター、ヒートショックプロモーター、SRαプロモーター等を挙げることができる。また、ヒトCMVのIE遺伝子のエンハンサーをプロモーターと共に用いてもよい。

【0084】宿主細胞としては、ヒトの細胞であるマムルバ (Mamula) 細胞、サル科の細胞であるCOS細胞、チャイニーズ・ハムスターの細胞であるCHO細胞、HBT5637 (特開昭63-299) 等を挙げることができる。組換えベクターの導入方法としては、動物細胞にDNAを導入する方法であればいずれも用いることができる。例えば、エレクトロポレーション法 (Cytotechnology, 3, 133 (1990))、リン酸カルシウム法 (特開平2-227075)、リポフェクション法 [Proc. Natl. Acad. Sci. USA, 84, 7413 (1987)] 等を挙げることができる。

【0085】昆虫細胞を宿主として用いる場合には、例えばカレント・プロトコールズ・イン・モレキュラー・バイオロジー・サブマニュアル-38 (1987-1997)、Baculovirus Expression Vectors, A Laboratory Manual, W. H. Freeman and Company, New York (1992)、Bio/Technology, 9, 47 (1988)等に記載された方法によっても、本発明のポリペプチドを発現させることができる。

【0086】即ち、組換え遺伝子導入ベクターおよびバキュロウイルスを昆虫細胞に共導入して昆虫細胞培養液中中に組換えウイルスを伝えた後、さらに組換えウイルスを昆虫細胞に感染させ、本発明のポリペプチドを発現させることができる。該方法において用いられる遺伝子導入ベクターとしては、例えば、pUL1392、pUL1393、pBacII (ともにInvitrogen社製) 等を挙げることができる。

Ex Cell 1405 (いずれもJRI Biosciences社製)、Grace's Insect Medium (Nature, 195, 788 (1962)) 等を用いることができる。培養は、通常pH6~7、25~30℃等の条件下で、1~5日間行う。また、培養中に必要に応じて、ゲンタマイシン等の抗生物質を培養地に添加してもよい。

【0098】植物細胞を宿主として得られた形質転換体は、細胞として、または植物の細胞や器官に分化させて培養することができる。形質転換体を培養する培地としては、一般に使用されているラシグ・アンド・スーグ(MS)培地、ホワイット(White)培地、またはこれら培地にオーキシン、サイトカイニン等、植物ホルモンを添加した培地等を用いることができる。培養は、通常pH5~9、20~40℃の条件下で3~60日間行う。また、培養中に必要に応じて、カナマイシン、ハイグロマイシン等の抗生物質を培地に添加してもよい。

【0099】本発明のポリペプチドの生産方法としては、宿主細胞内に生産させる方法、宿主細胞外に分泌させる方法、あるいは宿主細胞外膜上に生産させる方法があり、使用する宿主細胞や、生産させるポリペプチドの構造を変えることにより、該方法を選択することができる。本発明のポリペプチドが宿主細胞内あるいは宿主細胞外膜上に生産される場合、ポーソソンの方法 [Proc. Natl. Acad. Sci. USA, 86, 8227 (1989)、Genes Develop., 4, 1288 (1990)]、または特開平5-336963、W094/23021等に記載の方法を準用することにより、該ポリペプチドを宿主細胞外に積極的に分泌させることができる。

【0100】すなわち、遺伝子組換えの手法を用いて、本発明のポリペプチドの活性部位を含むポリペプチドの手前にシグナルペプチドを付加した形で発現させることにより、本発明のポリペプチドを宿主細胞外に積極的に分泌させることができる。また、特開平2-227075に記載されている方法に準じて、ジヒドロ葉緑素元酵素遺伝子等を用いた遺伝子増幅系を利用して生産量を上昇させることもできる。

【0101】さらに、遺伝子導入した動物または植物の細胞を再分化させることにより、遺伝子が導入された動物細胞 (トランスジェニック非ト動物) には植物体 (トランスジェニック植物) を造成し、これらの個体を用いて本発明のポリペプチドを製造することもできる。形質転換体が動物個体または植物個体の場合は、通常のの方法に従って、飼育または栽培し、該ポリペプチドを生産蓄積させ、動物個体または植物個体より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。

【0102】動物個体を用いて本発明のポリペプチドを製造する方法としては、例えば、知の方法 (American Journal of Clinical Nutrition, 53, 639S (1996)、Ame



たは透析することにより、該ポリペプチドを正常な立体構造に戻した後、上記と同様の単離精製法により該ポリペプチドの精製標品を得ることができる。

【0107】本発明のポリペプチドあるいはその誘導体等の誘導体が細胞外に分泌された場合には、培養上清に該ポリペプチドあるいはその誘導体付加体等の誘導体を回収することができる。即ち、該培養物を上記と同様の遠心分離等の手法により処理することにより可溶性成分を取得し、該可溶性成分から、上記と同様の単離精製法を用いることにより、精製標品を得ることができる。

【0108】また、本発明のポリペプチドは、Fmoc法（フルオレニルメチルオキシカルボニル法）、t Boc法（t-ブチルオキシカルボニル法）等の化学合成法によっても製造することができる。また、Advanced Chemical社、Perkin-Elmer社、Amersham Pharmacia Biotech社、Protein Tec hnology、Instrumental、Synthecell、Vega社、PerSeptive社は、島津製作所等のペプチド合成機を利用して化学合成することもできる。

【0109】4. 本発明のポリペプチドを認識する抗体の調製

本発明のポリペプチドまたは該ポリペプチドの部分断片ポリペプチドの精製標品、あるいは本発明のポリペプチドの一部のアミノ酸配列を有する合成ペプチドを抗原として用いることにより、ポリクロール抗体、モノクロール抗体等、本発明のポリペプチドを認識する抗体を調製することができる。

【0110】(1) ポリクロール抗体の作製

本発明のポリペプチドの全量または該ポリペプチドの部分断片ポリペプチドの精製標品、あるいは本発明のポリペプチドの一部のアミノ酸配列を有するペプチドを抗原として用い、適当なアジュバント（例えば、フロイントの完全アジュバント(Complete Freund's Adjuvant)または水酸化アルミニウムゲル、百日咳ワクチン等）とともに、動物の皮下、静脈内または腹腔内に投与することによりポリクロール抗体を作製することができる。

【0111】投与する動物として、ウサギ、ヤギ、3〜20週齢のラット、マウス、ハムスター等を用いることができる。該抗原の投与量は動物1匹当たり50〜100μgが好ましい。ペプチドを用いる場合は、ペプチドをスカジアヘモシアニン（keyhole limpet haemocyanin）や牛チログロブリン等のキャリア蛋白に共有結合させたものを抗原とするのが望ましい。抗原とするペプチドは、ペプチド合成機で合成することができる。

【0112】該抗原の投与は、1回目の投与の後、1〜2週間おきに3〜10回行う。各投与後、3〜7日目に腹腔静脈路より採血し、該血清が免疫に用いた抗原と反応することを酵素免疫測定法（酵素免疫測定法（ELISA法）：医学書院刊（1976年）、Antibodies-A Laboratory Manual、Cold Spring Harbor Laboratory 1988）等で確認する。

rican Journal of Clinical Nutrition, 63, 627S (1996)、Bio/Technology, 9, 830 (1991)) に準じて遺伝子を導入して遺伝した動物中に本発明のポリペプチドを生産する方法が挙げられる。

【0103】動物飼育の場合は、例えば、本発明のポリペプチドをコードするDNAを導入したトランスジェニック非ヒト動物を飼育し、該ポリペプチドを該動物中に生成・蓄積させ、該動物中より該ポリペプチドを採取することにより、該ポリペプチドを製造することができる。該動物中の蓄積物としては、例えば、該動物のミルク（特開昭63-309192）、卵等を挙げることができる。この際に用いられるプロモーターとしては、動物で発現できるものであればいずれも用いることができるが、例えば、乳腺細胞特異的なプロモーターであるαカゼインプロモーター、βカゼインプロモーター、βラクタログロブリンプロモーター、ホエー酸性プロテインプロモーター等が好適に用いられる。

【0104】植物体を用いて本発明のポリペプチドを製造する方法としては、例えば本発明のポリペプチドをコードするDNAを導入したトランスジェニック植物を公知の方法（組織培養, 20 (1994)、組織培養, 21 (1995)、Trends In Biotechnology, 15, 45 (1997)) に準じて栽培し、該ポリペプチドを該植物中に生成・蓄積させ、該植物中より該ポリペプチドを採取することにより、該ポリペプチドを生産する方法が挙げられる。

【0105】本発明の性質転換体により製造されたポリペプチドは、例えば本発明のポリペプチドが、細胞内に溶解状態で発現した場合には、培養終了後、細胞を遠心分離により回収し、水素過酸化にけん固後、超音波破砕、フレンチプレス、マントンゴウリンホモゲナイザー、ダイミル等により細胞を破砕し、無細胞抽出液を得る。該無細胞抽出液を遠心分離することにより得られる上清から、通常の酵素の単離精製法、即ち、溶媒抽出法、硫酸等による塩析法、脱塩法、有機溶媒による脱脂、ジエチルアミンエチル（DEAE）-セファロー、DIAIONHPA-75（三愛化成社製）等レジオンを用いた陰イオン交換クロマトグラフィー法、Sephacrose FF（Pharmacia社製）等のレジンをを用いた陽イオン交換クロマトグラフィー法、ブチルセアロース、フェニルセアロース等のレジンを用いた疎水性クロマトグラフィー法、分子篩を用いたゲル濾過法、アフィニティークロマトグラフィー法、クロマトフオーカシング法、等電気泳動気液動等の電気泳動法等の手法を単独あるいは組み合わせて用い、精製標品を得ることができる。

【0106】また、該ポリペプチドが細胞内に不溶性を形成して発現した場合は、同様に細胞を回収後破砕し、遠心分離を行うことにより、沈殿分としてポリペプチドの不溶性を回収する。回収したポリペプチドの不溶性をタンパク質変性剤で可溶化する。該可溶性液を希釈さ

う混合し、1, 200rpmで5分間遠心分離した後、上清を捨てる。

【0118】得られた沈殿成分の細胞群をよくほぐし、該細胞群に、攪拌しながら、37℃で、10<sup>6</sup>抗体産生細胞あたり、ポリエチレングリコール1000（PEG-1000）2g、MEM 2mlおよびジメチルスルホキシド（DMSO）0. 7mlを混合した溶液を0. 2〜1mlを数回添加する。

【0119】添加後、MEM培地を加えて全量が50mlになるように調整する。該懸濁液を900rpmで5分間遠心分離後、上清を捨て、得られた沈殿成分の細胞を、ゆるやかにほぐした後、メスベレットによる吸込み、吹出しでゆるやかにHAT培地（正格培地にヒポキサンチン（10<sup>-4</sup>mol/l）、チミジン（1. 5×10<sup>-5</sup>mol/l）およびアミンブテリン（4×10<sup>-4</sup>mol/l）を加えた培地）100ml中に懸濁する。

【0120】該懸濁液を96穴培養用プレートに100μl/穴ずつ分注し、5%CO<sub>2</sub>インキュベーター中、37℃で7〜14日間培養する。培養後、培養上清の一部をとりアンチボディーズ（Antibodies, A Laboratory manual, Cold Spring Harbor Laboratory, Chapter 14 (1988)) 等に述べられている酵素免疫測定法により、本発明のポリペプチドの部分断片ポリペプチドに特異的に反応するハイブリドーマを選別する。

【0121】酵素免疫測定法の具体的例として、以下の方法を挙げることができる。免疫の際、抗原に用いた本発明のポリペプチドの部分断片ポリペプチドを適当なプレートにコートし、ハイブリドーマ培養上清もしくは該述の(d)で得られる精製抗体を第一抗体として反応させ、さらに第二抗体としてビオチン、酵素、化学発光物質あるいは放射線化合物等で標識したラットまたは抗マウスIgMノグロブリン抗体を反応させた後に標識物質に応じた反応を行ない、本発明のポリペプチドに特異的に反応するものを本発明のモノクロール抗体を生産するハイブリドーマとして選択する。

【0122】該ハイブリドーマを用いて、膜希釈法によりクロニングを2回繰り返す（1回目は、HT培地（HAT培地からアミノプテリンを除いた培地）、2回（HAT培地からアミノプテリンを除いた培地）の配目は、正常培地を使用する）、安定して強い抗体を産生するめられたものを本発明のモノクロール抗体を生産するハイブリドーマ株として選択する。

(d) モノクロール抗体の調製

プリスタン処理（2, 6, 10, 14-テトラメチルペンタデカン（Pristane）0. 5ml）を腹腔内投与し、2週間飼育する）した8〜10週齢のマウスまたはヌードマウスに、(c)で取得した本発明のポリペプチドに対するモノクロール抗体産生ハイブリドーマ細胞5〜20×10<sup>6</sup>細胞/匹を腹腔内に注射する。10〜21日間でハイブリドーマは腹水産生する。

【0113】免疫に用いた抗原に対し、その血清が十分な抗体価を示した非ヒト哺乳動物より血清を取得し、該血清を分離、精製することによりポリクロール抗体を取得することができる。分離、精製する方法としては、遠心分離、40〜50%飽和硫酸アモンニウムによる塩析、カプリル酸沈殿（Antibodies, A Laboratory manual, Cold Spring Harbor Laboratory, (1988))、またはDEAE-セファロースカラム、陰イオン交換カラム、プロテインAまたはG-カラムあるいはゲル濾過カラム等を用いるクロマトグラフィー等を、単独または組み合わせて処理する方法が挙げられる。

【0114】(2) モノクロール抗体の作製

(a) 抗体産生細胞の調製

免疫に用いた本発明のポリペプチドの部分断片ポリペプチドに対し、その血清が十分な抗体価を示したラットを抗体産生細胞の供給源として供する。該抗体価を示したラットに抗原物質を最終投与した後3〜7日目に、脾臓を摘出する。

【0115】脾臓をMEM培地（日本製薬社製）中で細断し、ピンセットでよくほぐし、1, 200rpmで5分間遠心分離した後、上清を捨てる。得られた沈殿成分の脾細胞をトリス-塩化アンモニウム緩衝液（pH7. 6）5で1〜2分間処理し赤血球を除去した後、MEM培地で3回洗浄し、得られた脾細胞を抗体産生細胞として用いる。

【0116】(b) 骨髄細胞の調製

骨髄細胞としては、マウスまたはラットから取得した株化細胞を使用する。例えば、8-アザグアニン耐性マウス（BALB/c由来）骨髄細胞株P3-X63-A8-U1（以下、P3-U1と略す）（Curr. Topics. Microbiol. Immunol., 81, 1 (1978)、Europ. J. Immunol., 5, 511 (1976))、SP2/O-Ag14（SP2）（Nature, 276, 269 (1978))、P3-X63-A8653（653）（J. Immunol., 123, 1548 (1979)）、P3-X63-A8（X63）（Nature, 256, 495 (1975)) 等を用いることができる。これらの細胞株は、8-アザグアニン培地（RPMI-1640培地にグルタミン（5×10<sup>-5</sup>mol/l）、2-メルカプトエタノール（5×10<sup>-5</sup>mol/l）、ジェンタマイシン（10μg/ml）および胎胎血清（FCS）（CSL社製）10%を加えた培地（以下、正常培地という）に、さらに8-アザグアニン（15μg/ml）を加えた培地で維持するが、細胞割合の3〜4日前に正常培地で培養し、融合には該細胞を2×10<sup>6</sup>/個以上用いる。

【0117】(c) ハイブリドーマの作製

(b)で取得した抗体産生細胞と(b)で取得した骨髄細胞をMEM培地またはPBS（リン酸二ナトリウム1. 83g、リン酸一カリウム0. 21g、塩基7. 65g、蒸留水1リットル、pH7. 2）でよく洗浄し、細胞数が、抗体産生細胞：骨髄細胞=5〜10:1になるよ

【0123】胚頭水抽出したマウスから腹水を採取し、3.000rpmで5分間遠心分離して固形分を除去する。得られた上清より、ポリクローナルで用いた方法と同様の方法でモノクローナル抗体を精製し、取得することができる。抗体のサブクラス決定は、マウスモノクローナル抗体タイプベンジキットまたはラットモノクローナル抗体タイプベンジキットを用いて行う。タンパク質量は、ローリー法あるいは280nmでの吸光度より算出する。

【0124】5.本発明のポリペプチドを生産する組換えウイルスベクターの調製法  
以下に、本発明のポリペプチドを特定のヒト組織内で生産するための組換えウイルスベクターの調製法について述べる。本発明のDNAの完全長cDNAをもとに、必要に応じて、該ポリペプチドをコードする部分を含む適当な長さのDNA断片を調製する。

【0125】完全長cDNA、あるいは該DNA断片をウイルスベクター内のプロモーターの下流に挿入することにより、組換えウイルスベクターを造成する。RNA断片は、2本鎖の他、ウイルスベクターの種類に応じて、センス鎖若しくはアンチセンス鎖のどちらか一方の本鎖を選択する。例えば、レトロウイルスベクターの場合は、センス鎖に相同するRNAを、センダイウイルスベクターの場合は、逆にアンチセンス鎖に相同するRNAを選択する。

【0126】該組換えウイルスベクターを、該ベクターに適合したパッケージング細胞に導入する。パッケージング細胞はウイルスのパッケージングに必要なポリペプチドをコードするDNAの少なくとも1つを欠損している組換えウイルスベクターの欠損部分をポリペプチドで補給できる細胞は全て用いることができる。例えばヒト腎臓由来のHEK293細胞、マウス繊維芽細胞NIH3T3等を用いることができる。パッケージング細胞で補給するポリペプチドとしては、レトロウイルスベクターの場合はマウスレトロウイルス由来のgag、pol、env等のエンベロペプチドが、レンウイルスベクターの場合はHIVウイルス由来のgag、pol、env、vpr、vpu、vif、tat、rev、nef等のポリペプチド、アデノウイルスベクターの場合はアデノウイルス由来のE1A、E1B等のポリペプチドが、アデノ随伴ウイルスの場合はRep(p5、p19、p40)、VP(Cap)等のポリペプチドが、センダイウイルスの場合はNP、p/C、L、M、F、HN等のポリペプチドが挙げられる。

【0127】ウイルスベクターとしては上記パッケージ

ング細胞において組換えウイルスが生産でき、標的細胞で本発明のDNAを転写できる位置にプロモーターを含有しているものが用いられる。プラスミドベクターとしてはpRC[Proc. Natl. Acad. Sci. USA, 92, 6733-6737 (1995)]、pBabePuro[Nucleic Acids Res., 19, 3587-3596 (1990)]、IL-CG-CLC-CS-CG, CLC[Journal of Virology, 72, 8150-8157 (1998)]、pDext[*Nucleic Acids Res.*, 23, 3816-3821 (1995)]等が用いられる。【0128】プロモーターとしては、ヒト組織中で発現できるものであればいずれも用いることができ、例えば、サイトメガロウイルス(ヒトCMV)のIE(Immediate early)遺伝子のプロモーター、SV40の初期プロモーター、レトロウイルスのプロモーター、メタロチオネインプロモーター、ヒートショックタンパク質プロモーター、SRαプロモーター等を挙げることで、また、ヒトCMVのIE遺伝子のエンハンサーをプロモーターと共に用いてもよい。

【0129】パッケージング細胞への組換えウイルスベクターの導入法としては、例えば、リン酸カルシウム法(特開平2-227075号公報)、リポフェクション法[Proc. Natl. Acad. Sci. USA, 84, 7413 (1987)]等を挙げることができる。

6. 本発明のDNA、ポリペプチドまたは抗体の利用  
(1) 本発明のDNAの発現を抽出する方法  
本発明のDNAを用いて、抗体における本発明のDNAのmRNA発現、該mRNAの構造変化を抽出することができる。

【0130】抗体としては、本発明のDNAの発現変化が原因となっている疾患を有する患者ならびに健康者より取得した組織、血清、唾液等の生体試料、該生体試料から細胞を取得しては細胞質内の適当な場中で培養した初代培養細胞試料、または生体試料から取得した組織を、パラフィンあるいはクリオスタット切片として凍結し、パラフィンあるいはクリオスタット切片として凍結したもの等から取得したmRNAあるいは全RNA等が用いられる(以後、該mRNAおよび全RNAを抗体由来RNAと称する)。

【0131】抽出する方法としては、例えば(1)ノーザンブロット法(2)in situハイブリダイゼーション法、(3)定量的PCR法、(4)デファレンシャル・ハイブリダイゼーション法[Trends in Genetics 7, 31 (1991)]、(5)DNAチップ法[Genome Research h, 6, 639, (1996)]、(6)RNAセットアップ法等の方法等が挙げられる。以下、各抽出法について詳述する。

【0132】①ノーザンブロット法  
抗体由来RNAをゲル電気泳動で分離後、ナイロンフィルター等の支持体に転写する。転写後、本発明のDNAより調製した探針プローブを用いて、ハイブリダイゼーションならびに洗浄を行う。洗浄後、該プローブと特異的に結合したRNAの発現を抽出する。健康者と患者

由来の抗体RNAについて該検出結果を比較することにより、該RNAの発現量ならびに構造の変化を抽出することができる。ハイブリダイゼーションの際には、プローブと抗体由来RNA中の目的とするmRNAが安定なハイブリッドを形成する条件でインキュベーションする。偽陽性を防ぐためには、ハイブリダイゼーションならびに洗浄工程をモレキュラー・クロニング第2版に記載の方法に準じて高ストリンジェントな条件で行うことが望ましい。

【0133】ノーザンブロット法に用いる探針プローブは、例えば、公知の方法(ニック・トランスレーション、ランダム・プライミングまたはキナージング)により放射性同位体、ビオチン、蛍光基、化学発光基等、本発明のDNAあるいは該DNAの配列から設計したオリゴヌクレオチドに取り込ませることによって調製できる。探針プローブのmRNAへの結合量は該mRNAの発現量を反映することから、結合した探針プローブの量を定量的に測定することによって該mRNAの発現量を定量的に測定することができる。また、探針プローブが結合するフィルター上の部位を分析することで、該mRNAの構造変化を知ることができる。

【0134】②in situハイブリダイゼーション法  
生体から取得した組織をパラフィンあるいはクリオスタット切片として凍結し得られた抗体、および①記載の探針プローブを用いてハイブリダイゼーションならびに洗浄工程を行う。洗浄後、①と同様の方法により該プローブと特異的に結合したmRNAの発現量を抽出することができる。in situハイブリダイゼーション法で、偽陽性を防ぐために、ハイブリダイゼーションならびに洗浄工程をカレン・プロット・コールズ・イン・モレキュラー・バイオロジー等に記載されている方法に準じて高ストリンジェントな条件で行うことが望ましい。

【0135】③定量的PCR法  
抗体由来RNA、オリゴdTプライマーまたはランダムプライマー、および逆転写酵素を用い、cDNAを合成することに基づいた方法を用いることにより目的とするRNAを抽出することができる(以後、該cDNAを抗体由来cDNAと称する)。抗体由来RNAがmRNAの場合には、上記①のいずれのプライマーも用いることができるが、該抗体由来RNAが全RNAである場合は、オリゴdTプライマーを用いることが必要である。

【0136】定量的PCR法では、抗体由来cDNAをテンプレートとし本発明のDNAが有する塩基配列に基づき設計したプライマーを用いてPCRを行うことで、特定のmRNA由来のDNA断片が増幅される。該増幅DNA断片の量は該mRNAの発現量を反映することから、アクチンやG3PDH(glyceraldehyde 3-phosphate dehydrogenase)等をコードするDNAを内部コントロールとして置くことで該mRNAの量を定量的に測定可能である。また、該増幅DNA断片をゲル電気泳動に

より分離することで、該mRNAの構造の変化を知ることができる。本発明では、標的配列を特異的にかつ効率的に増幅する適当なプライマーを用いることが望ましい。適当なプライマーは、プライマー間の結合やプライマー内の結合を起さず、アニーリング温度で標的cDNAと特異的に結合して、変性条件で標的cDNAからはずれる等の条件に基づき設計することができる。増幅DNA断片の定量は増幅産物が指数関数的に増加しているPCR反応の、各反応ごとに生産される該増幅DNA断片を回収してゲル電気泳動で定量的分析することで知ることができる。

【0137】④デファレンシャル・ハイブリダイゼーション法およびDNAチップ法  
⑤に記載された方法で調製した抗体由来cDNAをプローブとして、本発明のDNAを固定化させたフィルターあるいはスライドガラスやシリコン等の基盤に対してハイブリダイゼーションならびに洗浄を行う。洗浄後、本発明のDNAと特異的に結合したcDNA量を測定することにより該cDNA由来のmRNAの発現量の変動を抽出することができる。デファレンシャル・ハイブリダイゼーション法およびDNAチップ法のいずれの方法もフィルターあるいは基盤上にアクチンやG3PDH等の内部コントロールを固定化することで、対照抗体と標的抗体の間での該mRNAの発現の違いを正確に検出することができる。また対照抗体と標的抗体由来のRNAをもとにそれぞれ異なる探針dNTPを用いて探針cDNAを合成を行い、1枚のフィルターあるいは1枚の基盤に二つの探針cDNAプローブを同時にハイブリダイゼーションすることによって正確な該mRNAの発現量の定量的を行うことができる。

【0138】⑤RNAセットアップ法  
本発明のDNAの3'端にT7プロモーター、SP6プロモーター等のプロモーター配列を結合し、RNAポリメラーゼを用いたin vitroの転写系により探針cDNAを合成し、探針cDNAと抗体由来RNAを合成する。該探針cDNAと抗体由来RNAを結合させた後、RNA-HAハイブリッドを形成させた後、RNAを消化し、消滅から保護されたRNA断片をゲル電気泳動によりバンドを形成させ検出する。得られたバンドを定量的に測定することで、上記探針cDNAと抗体由来RNAの発現量を定量的に測定することができる。

【0139】尚、①～⑤のいずれかに記載した方法に用いられるDNAとしては、例えば配列番号6～10のいずれかで表される塩基配列を有するDNAもしくはそれらから得られるDNA断片等が挙げられる。また、当該方法による検出に供する抗体としては、アレクサー、アトピー、喘息、花粉症、気管支炎、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、

【0142】①ポリアクリルアミドゲル電気泳動によるヘテロ二本鎖検出法

検体由来DNAあるいは検体由来cDNAをテンプレートに、該DNAを配列番号6〜10のいずれかに記載の塩基配列に基づき設計したプライマーにより、2本鎖pよりも小さいDNA断片として増幅する。本発明のDNAおよび被検者由来の該増幅DNA断片を用い、各々の増幅DNA断片による2本鎖形成処理を常法により行う。処理後、ポリアクリルアミドゲル電気泳動を行う。該DNAの変異によりヘテロ二本鎖が形成された場合は、変異を持たないホモ二本鎖よりも移動度が遅く、それらはホモ二本鎖とは別のバンドとして検出することができる。特製のゲル (Hydro-link, MDAなど) を用いた方が分離度はよい。200bpよりも小さい断片の検出ならば、挿入、欠失、ほとんどの1塩基置換を検出可能である。ヘテロ二本鎖解析は、次に述べる一本鎖コンフォメーション多型解析と組み合わせれば1枚のゲルで行うことが望ましい。

【0143】②一本鎖コンフォメーション多型解析法  
一本鎖コンフォメーション多型解析 (SSCP解析; single strand conformation polymorphism analysis) では、検体由来DNAあるいは検体由来cDNAをテンプレートに、配列番号6〜10のいずれかに記載の塩基配列に基づき設計したプライマーにより、200bpよりも小さい断片として増幅した該DNAを変性後、変異性ポリアクリルアミドゲル中で電気泳動する。DNA増幅を行う際にプライマーを放射同位体あるいは蛍光色素で標識し、該標識を指標とするか、または未標識の増幅産物を電気泳動後、銀染色することにより、増幅した該DNAをバンドとして検出することができる。本発明のDNA由来の増幅DNA断片と、被検者由来のものと同様に電気泳動することにより、変異を持った断片を移動度の遅いから検出できる。

【0144】③ミスマッチの化学的切断法  
ミスマッチの化学的切断法 (CCM法) では、検体由来DNAあるいは検体由来cDNAをテンプレートに、該DNAを配列番号6〜10のいずれかに記載の塩基配列に基づき設計したプライマーで増幅したDNA断片を、本発明のDNAに放射性同位体あるいは蛍光色素をとり込ませた増幅DNAとハイブリダイズさせ、四酸化オースミウムで処理することでミスマッチしている場所のDNAの一方の鎖を切断させ変異を検出することができる。CCM法は最も感度の高い検出法の一つであり、エラーの低さの検体にも適応できる。

【0145】④ミスマッチの酵素的切断法  
上記四酸化オースミウムの代わりにT4ファージソリベースとエンドヌクレアーゼV111のような細菌内でミスマッチの修復に関与する酵素とRNaseAと組み合わせることで、酵素的にミスマッチを切断することもできる。

エンドトキシンショック、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、瘰癧、各種脳脊髄炎、うつ病、心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、パーキンソン病、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性性腫瘍等の異常な増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な増殖を伴う疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群 (SIRS: systemic inflammatory response syndrome)、成人呼吸器 distress 候群 (ARDS: adult respiratory distress syndrome) 等の疾患が挙げられる。当該検出方法により本発明のDNAの発現を検出することで、上記疾患の診断に利用することができる。

【0140】(2) 本発明のDNAの変異を検出する方法

以下、被検者における本発明のDNAの変異の有無を検出する方法について述べる。被検者における該DNAの変異は本発明のDNAと配列番号により直接比較することにより検出することができる。被検者から、組織、血清、唾液等のヒト生体試料あるいは、被生体試料から樹立した初代培養細胞由来の試料を集め、該生体試料あるいは該初代培養細胞由来試料からDNAを抽出する (以下、該DNAを検体由来DNAと称する)。また、該試料由来のmRNAより常法によりcDNAを取得する (以下、該cDNAを検体由来cDNAと称する)。該検体由来DNAまたはcDNAを解型とし、本発明のDNAが有する塩基配列に基づき設計したプライマーを用いてPCR法等によりDNAを増幅する。得られた増幅DNAを試料DNAとして用いる。

【0141】増幅DNAに変異があるかどうかを検出する方法として、野生型対立遺伝子を有するDNA鎖と変異対立遺伝子を有するDNA鎖とのハイブリダイズにより形成されるヘテロ二本鎖を検出する方法を用いることができる。ヘテロ二本鎖を検出する方法には、①ポリアクリルアミドゲル電気泳動によるヘテロ二本鎖検出法 (Trends Genet., 7, 5 (1991))、②一本鎖コンフォメーション多型解析法 (Genomics, 16, 325-332 (1993))、③ミスマッチの化学的切断法 (CCM, chemical cleavage of mismatches) (Human Molecular Genetics (1996), Tom Strachan and Andrew P. Read (B105 Scientific Publications Li, aited)), ④ミスマッチの酵素的切断法 (Nature Genetics, 9, 103-104 (1996))、⑤変異性ゲル電気泳動法 (Mutat. Res., 288, 103-112 (1993))、⑥タンパク質短縮試験法 (protein truncation test: PTT法) (Genomics, 20, 1-4 (1994)) 等の方法が挙げられる。以下、上記方法について説明する。

ポリダイゼインシジョンのプロープとして用いることにより、クローン化することができる。非コード領域における変異は上述のいずれかの方法に準じて探索することができる。

【0150】見い出された変異は、Handbook of Human Genetics Linkage, The John Hop Kins University Press, Baltimore (1994) に記載された方法に従って統計処理を行うことで、疾患との相関があるSNPs (シングル・ヌクレオチド・ポリモルフィズム) として同定することができる。上記変異を検出する方法で診断可能な被験者としては、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、瘰癧、各種脳脊髄炎、うつ病、心不全、外傷性脳損傷、炎症性腸疾患等の感染や炎症を伴う疾患、パーキンソン病、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性性腫瘍等の異常な細胞増殖を伴う疾患、関節リウマチ、変形性関節炎等の異常な増殖を伴う疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経細胞の障害に基づく疾患、動脈硬化・再狭窄などの平滑筋細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性炎症反応症候群 (SIRS: systemic inflammatory response syndrome)、成人呼吸器 distress 候群 (ARDS: adult respiratory distress syndrome) 等のいずれかの疾患を有する者を挙げることができる。

【0151】(3) 本発明のDNAまたはオリゴヌクレオチドを用いて本発明のポリペプチドをコードするDNAの転写または翻訳を抑制する方法  
アンチセンスRNA/DNA技術 (ハイオサイエンスとインダストリー, 50, 322 (1992)、化学, 46, 681 (1993)、Biotechnology, 9, 358 (1992)、Trends in Biotechnology, 10, 87 (1992)、Trends in Biotechnology, 10, 152 (1992)、細胞工学, 16, 1463 (1997))、トリプル・ヘリックス技術 (Trends in Biotechnology, 10, 132 (1992)) 等により、本発明のDNAを利用して本発明のポリペプチドをコードするDNAの転写または翻訳を抑制することができる。例えば、本発明のDNAまたはオリゴヌクレオチドを、本発明のポリペプチドを発現できる系 (生体を含む) に共存させ、該ポリペプチドの発現を転写・翻訳レベルで抑制できる。

【0152】該抑制方法は、アレルギー、アトピー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エンドトキシンショック、敗血症、微生物感染、慢性型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、乾癬、瘰癧、各種脳脊髄炎、うつ病、心不全、







Tyr Ser Lys Leu Arg Ala Gln Asn Gln Val Leu Lys Lys Gly Val Val  
20 25 30  
Asp Glu Gln Ala Asn Ser Ala Ala Leu Lys Glu Gln Leu Lys Met Lys  
35 40 45  
Asp Gln Ser Leu Arg Lys Leu Gln Gln Glu Met Asp Ser Leu Thr Phe  
50 55 60  
Arg Asn Leu Leu Leu Ala Lys Arg Val Glu Leu Leu Gln Asp Gln Leu  
65 70 75 80  
Ala Leu Ser Glu Arg Gly Lys Lys Asn Lys Lys Ser Gly Glu Ser  
85 90 95  
Ser Ser Gln Leu Ser Gln Gln Gln Lys Ser Val Phe Asp Glu Asp Leu  
100 105 110  
Gln Lys Lys Ile Glu Glu Asn Glu Arg Leu His Ile Gln Phe Glu  
115 120 125  
Ala Asp Glu Gln His Lys His Val Glu Ala Glu Leu Arg Ser Arg Leu  
130 135 140  
Ala Thr Leu Glu Thr Glu Ala Ala Gln His Gln Ala Val Val Asp Gly  
145 150 155 160  
Leu Thr Arg Lys Tyr Met Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys  
165 170 175  
Ala Lys Leu Glu Val Lys Ser Gln Thr Leu Glu Lys Glu Ala Lys Glu  
180 185 190  
Cys Arg Leu Arg Thr Glu Glu Cys Gln Leu Gln Leu Lys Thr Leu His  
195 200 205  
Glu Asp Leu Ser Gly Arg Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu  
210 215 220  
Lys Val Pro Phe Asn Asp Thr Lys Tyr Ser Gln Tyr Asn Ala Leu Asn  
225 230 235 240  
Val Pro Leu His Asn Arg Arg His Gln Leu Lys Met Arg Asp Ile Ala  
245 250 255  
Gly Gln Ala Leu Ala Phe Val Gln Asp Leu Val Thr Ala Leu Leu Asn  
260 265 270  
Phe His Thr Tyr Thr Glu Gln Arg Ile Gln Ile Phe Pro Val Asp Ser  
275 280 285  
Ala Ile Asp Thr Ile Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu  
290 295 300  
His Glu Asn Ala Ser Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His  
305 310 315 320  
Leu Phe Glu Ser Ile Thr Glu Asp Thr Val Thr Val Leu Glu Thr Thr  
325 330 335  
Val Lys Leu Lys Thr Phe Ser Glu His Leu Thr Ser Tyr Ile Cys Phe  
340 345 350  
Leu Arg Lys Ile Leu Pro Tyr Gln Leu Lys Ser Leu Glu Glu Cys  
355 360 365  
Glu Ser Ser Leu Cys Thr Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu  
370 375 380  
Ser Gln Asp Met Lys Met Thr Ala Val Phe Glu Lys Leu Gln Thr  
385 390 395 400

細胞の障害に基づく疾患、動脈硬化・再狭窄等の平滑筋  
細胞の異常な分化増殖を伴う疾患、多臓器不全、全身性  
炎症反応症候群 (SIRS: systemic inflammatory r  
esponsesyndrome)、成人呼吸器迫症候群 (ARDS: a  
dult respiratory distress syndrome) 等の治療薬の探  
索、開殖に有用なポリペプチド、該ポリペプチドをコー  
ドするDNA、該DNAのアナセンズDNA/RN  
A、該DNAを用いた遺伝子治療、該ポリペプチドを認  
識する抗体、該ポリペプチドの活性上昇変異体、該ポリ  
ペプチドのドミナントネグティブ変異体、およびこれら  
の利用法を提供することができる。  
【0197】  
【配列表フリーテキスト】  
配列番号11ー人工配列の説明：合成DNA (オリゴキヤ  
ップリンカー配列)  
配列番号12ー人工配列の説明：合成DNA (オリゴデブ  
ライマー配列)  
配列番号13ー人工配列の説明：合成DNA (5'末端側の  
センスプライマー配列)  
配列番号14ー人工配列の説明：合成DNA (3'末端側の  
アンチセンスプライマー配列)  
配列番号15ー人工配列の説明 (転写因子NF-κ結合配  
列)  
配列番号16ー人工配列の説明：合成DNA (組織発現分  
布を検討した合成プライマー配列)  
配列番号17ー人工配列の説明：合成DNA  
配列番号18ー人工配列の説明：合成DNA  
配列番号19ー人工配列の説明：合成DNA  
配列番号20ー人工配列の説明：合成DNA  
配列番号21ー人工配列の説明：合成DNA  
配列番号22ー人工配列の説明：合成DNA  
配列番号23ー人工配列の説明：合成DNA  
【0198】  
【配列表】

用した。PCR用プライマーとしては、COL0327  
9からの塩基配列情報に基づいた配列番号16および1  
7、COL06772からの塩基配列情報に基づいた配  
列番号18および19、ADKA01604からの塩基  
配列情報に基づいた配列番号20および21、ADSU  
0701からの塩基配列情報に基づいた配列番号22  
および23に記載の合成DNAを用いた。PCR反応  
は、ニッポンジーン社製のRecombinant Taq DNA Polyme  
rase (GeneTaq) と添付の10×Gene Taq Universal Bu  
fferおよび2.5 mmol/l dNTP Mixtureを用  
いて、説明書に従って行った。MJ RESEARCH社  
製のサーマル・サイクラーを用いて、94℃で30秒  
間、60℃で1分間、72℃で2分間の反応を26〜3  
0サイクル行った。反応液をアガロースゲル電気泳動法  
およびエチジウムブロマイド染色により解析した。  
【0195】結果を図1〜4に示す。COL0327  
9、COL06772、ADKA01604、ADSU  
07010の各クローンに認められる本発明のDNA  
は、各クローン、各臓器によって強調の差はあるもの  
の、検出した35個全ての臓器で発現していた。

【0196】  
【発明の効果】本発明によれば、アレルギー、アトピ  
ー、喘息、花粉症、気道過敏、自己免疫疾患、移植片対  
宿主疾患等の異常な免疫細胞の活性化を伴う疾患、エン  
ドトキシンショック、敗血症、微生物感染、慢性B型肝炎、慢性C型肝炎、インスリン依存性・非依存性糖尿病、糸球体腎炎、外傷性脳損傷、乾癪、痛風、各種脳脊髄炎、うつ血性心不全、炎症性腸疾患等の感染や炎症を伴う疾患、バネキッドリンパ腫、ホジキン病、各種リンパ腫、成人T細胞白血病、悪性腫瘍等の異常な細胞増殖を伴う疾患、慢性胆嚢リウマチ、変形性関節炎等の異常な線維芽細胞や滑膜組織の活性化を伴う疾患、エイズ等のウイルス性疾患、虚血性脳疾患の神経細胞の障害に基づく疾患、アルツハイマー病、パーキンソン病等の神経

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81

62

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Ser Thr Phe Ser Glu Asn Glu Tyr Asp Ala Ser Trp Ser Pro Ser Trp  
35 40 45  
Val Met Trp Leu Glu Leu Pro Ser Thr Leu His Ser Cys His Asp Ile  
50 55 60  
Val Leu Arg Arg Ser Tyr Leu Gly Ser Trp Gly Phe Ser Ile Val Gly  
65 70 75 80  
Gly Tyr Glu Glu Asn His Thr Asn Gln Pro Phe Phe Ile Lys Thr Ile  
85 90 95  
Val Leu Gly Thr Pro Ala Tyr Tyr Asp Gly Arg Leu Lys Cys Gly Asp  
100 105 110  
Met Ile Val Ala Val Asn Gly Leu Ser Thr Val Gly Met Ser His Ser  
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Val Ile Cys Trp Pro Gly Ser Leu Val  
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[0201]

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Val Pro Val Arg Thr Lys Arg Phe Thr Pro Pro Ile Tyr Gln Pro  
35 40 45  
Lys Phe Lys Thr Glu Lys Glu Phe Met Gln His Ala Arg Lys Ala Gly  
50 55 60  
Leu Val Ile Pro Pro Glu Lys Ser Asp Arg Ser Ile His Leu Ala Cys  
65 70 75 80  
Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro Pro Glu Gly Asp Ala Arg  
85 90 95  
Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile Glu Arg Thr Glu Met  
100 105 110  
Lys Lys Thr Met Ala Ser Gln Val Ser Ile Arg Arg Ile Lys Asp Tyr  
115 120 125  
Asp Ala Asn Phe Lys Ile Lys Asp Phe Pro Gly Lys Ala Lys Asp Ile

80

59

Tyr Ile Ala Leu Leu Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu  
405 410 415  
Arg Thr Asn Tyr Ser Ser Val Leu Thr Asn Val Gly Ala Ala Leu His  
420 425 430  
Gly Phe His Asp Val Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys  
435 440 445  
Ala Ala Ile Glu His Glu Leu Pro Thr Ala Thr Gln Lys Leu Ile Thr  
450 455 460  
Thr Asn Asp Cys Ile Leu Ser Ser Val Val Ala Leu Thr Asn Gly Ala  
465 470 475 480  
Gly Lys Ile Ala Ser Phe Ser Ser Asn Asn Leu Asp Tyr Phe Ile Ala  
485 490 495  
Ser Leu Ser Tyr Gly Pro Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu  
500 505 510  
Ser Ala Glu Cys Met Leu Gln Tyr Lys Lys Ala Ala Tyr Met  
515 520 525  
Lys Ser Leu Arg Lys Pro Leu Leu Glu Ser Val Pro Tyr Glu Glu Ala  
530 535 540  
Leu Ala Asn Arg Arg Ile Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly  
545 550 555 560  
Leu Ala Gln Val Gln Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu  
565 570 575  
Gln Glu Lys Glu His Trp Met Leu Glu Ala Gln Leu Ala Lys Ile Lys  
580 585 590  
Leu Glu Lys Glu Asn Gln Arg Ile Ala Asp Lys Leu Lys Asn Thr Gly  
595 600 605  
Ser Ala Gln Leu Val Gly Leu Ala Gln Glu Asn Ala Val Ser Asn  
610 615 620  
Thr Ala Gly Gln Asp Glu Ala Thr Ala Lys Ala Val Leu Glu Pro Ile  
625 630 635 640  
Gln Ser Thr Ser Leu Ile Gly Thr Leu Thr Arg Thr Ser Asp Ser Glu  
645 650 655  
Val Pro Asp Val Glu Ser Arg Glu Asp Leu Ile Lys Asn His Tyr Met  
660 665 670  
Ala Arg Ile Val Glu Leu Thr Ser Gln Leu Leu Ala Asp Ser Lys  
675 680 685  
Ser Val His Phe Tyr Ala Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala  
690 695 700  
Leu Ala Glu Lys Ser Lys Glu Ala Leu Thr Glu Glu Met Lys Leu Ala  
705 710 715 720  
Ser Gln Asn Ile Ser Arg Leu Gln Asp Glu Thr Thr Thr Lys Arg  
725 730 735  
Ser Tyr Glu Asp Gln Leu Ser Met Met Ser Asp His Leu Cys Ser Met  
740 745 750  
Asn Glu Thr Leu Ser Lys Gln Arg Glu Glu Ile Asp Thr Leu Lys Met  
755 760 765  
Ser Ser Lys Gly Asn Ser Lys Lys Asn Lys Ser Arg  
770 775 780

[0200]

50

63

64

130 135 140  
Phe Ile Glu Ala His Leu Cys Leu Asn Ser Asp His Asp Arg Leu  
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His Thr Leu Val Thr Glu His Cys Phe Pro Asp Met Thr Trp Asp Ile  
165 170 175  
Lys Tyr Lys Thr Val Arg Trp Ser Phe Val Glu Ser Leu Glu Pro Ser  
180 185 190  
His Val Val Cln Val Arg Cys Ser Ser Met Met Asn Cln Gly Asn Val  
195 200 205  
Tyr Gly Cln Ile Thr Val Arg Met His Thr Arg Cln Thr Leu Ala Ile  
210 215 220  
Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly Cln Glu Asp Val Pro Lys  
225 230 235 240  
Asp Val Leu Glu Tyr Val Val Phe Glu Lys Cln Leu Thr Asn Pro Tyr  
245 250 255  
Gly Ser Trp Arg Met His Thr Lys Ile Val Pro Pro Trp Ala Pro  
260 265 270  
Lys Cln Pro Ile Leu Lys Thr Val Met Ile Pro Gly Pro Cln Leu Lys  
275 280 285  
Pro Glu Glu Tyr Glu Glu Ala Cln Gly Glu Ala Cln Lys Pro Cln  
290 295 300  
Leu Ala 305  
  
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20 25 30  
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35 40 45  
Glu Ala Lys Val Thr Ser Ala Cys Cln Ala Leu Pro Pro Val Glu Leu  
50 55 60  
Arg Arg Asn Thr Ala Pro Val Arg Arg Ile Glu His Leu Gly Ser Thr  
65 70 75 80  
Lys Ser Leu Asn His Ser Lys Cln Arg Ser Thr Leu Pro Arg Ser Phe  
85 90 95  
Ser Leu Asp Pro Leu Met Glu Arg Arg Trp Asp Leu Asp Leu Thr Tyr  
100 105 110  
Val Thr Glu Arg Ile Leu Ala Ala Ala Phe Pro Ala Arg Pro Asp Glu  
115 120 125  
Cln Arg His Arg Gly His Leu Arg Glu Leu Ala His Val Leu Cln Ser  
130 135 140  
Lys His Arg Lys Tyr Leu Leu Phe Asn Leu Ser Glu Lys Arg His

【0202】

65

66

145 150 155 160  
Asp Leu Thr Arg Leu Asn Pro Lys Val Cln Asp Phe Gly Trp Pro Glu  
165 170 175  
Leu His Ala Pro Pro Leu Asp Lys Leu Cys Ser Ile Cys Lys Ala Met  
180 185 190  
Glu Thr Trp Leu Ser Ala Asp Pro Cln His Val Val Val Leu Tyr Cys  
195 200 205  
Lys Val Gly Cln Asp Leu Gly Phe Pro Gly Ala Trp Arg Phe Cln Val  
210 215 220  
Ser Leu Glu Leu Pro Asp Pro His Pro Cys Leu Ser Val Cys Cln Gly  
225 230 235 240  
Asn Lys Gly Lys Leu Gly Val Ile Val Ser Ala Tyr Met His Tyr Ser  
245 250 255  
Lys Ile Ser Ala Gly 260  
  
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Glu Glu Cys Cln Leu Cln Leu Lys Thr Leu His Glu Asp Leu Ser Gly  
35 40 45  
Arg Leu Glu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn  
50 55 60  
Asp Thr Lys Tyr Ser Arg Tyr Asn Ala Leu Asn Val Pro Leu His Asn  
65 70 75 80  
Arg Arg His Cln Leu Lys Met Arg Asp Ile Ala Gly Cln Ala Leu Ala  
85 90 95  
Phe Val Cln Asp Leu Val Thr Ala Leu Leu Asn Phe His Thr Tyr Thr  
100 105 110  
Glu Cln Arg Ile Cln Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile  
115 120 125  
Ser Pro Leu Asn Cln Lys Phe Ser Cln Tyr Leu His Glu Asn Ala Ser  
130 135 140  
Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile  
145 150 155 160  
Thr Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Thr  
165 170 175  
Phe Ser Glu His Leu Thr Ser Tyr Ile Cys Phe Leu Arg Lys Ile Leu  
180 185 190  
Pro Tyr Cln Leu Lys Ser Leu Glu Glu Cys Glu Ser Ser Leu Cys  
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Thr Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Cln Asp Met Lys  
210 215 220

【0203】

67

(35)

68

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225 230 235 240  
Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Ser  
245 250 255  
Ser Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val  
260 265 270  
Met Lys Asp Ile Ser Lys His Tyr Ser Glu Lys Ala Ala Ile Glu His  
275 280 285  
Glu Leu Pro Thr Ala Thr Glu Lys Leu Ile Thr Thr Asn Asp Cys Ile  
290 295 300  
Leu Ser Ser Val Val Ala Ser Thr Asn Gly Ala Gly Lys Ile Ala Ser  
305 310 315 320  
Phe Phe Ser Asn Asn Leu Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly  
325 330 335  
Pro Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met  
340 345 350  
Leu Glu Tyr Lys Lys Ala Ala Tyr Met Lys Ser Leu Arg Lys  
355 360 365  
Pro Leu Leu Glu Ser Val Pro Tyr Glu Glu Ala Leu Ala Asn Arg Arg  
370 375 380  
Ile Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Glu Glu Val  
385 390 395 400  
Glu Glu Ser Leu Glu Lys Ile Ser Lys Leu Glu Glu Glu Lys Glu His  
405 410 415  
Trp Met Leu Glu Ala Glu Leu Lys Ile Lys Leu Glu Lys Glu Asn  
420 425 430  
Glu Arg Ile Ala Asp Lys Leu Lys Asn Thr Gly Ser Ala Glu Leu Val  
435 440 445  
Gly Leu Ala Glu Glu Asn Ala Val Ser Asn Thr Ala Gly Glu Asp  
450 455 460  
Glu Ala Thr Ala Lys Ala Val Leu Glu Pro Ile Glu Ser Thr Ser Leu  
465 470 475 480  
Ile Gly Thr Leu Thr Arg Thr Ser Asp Ser Glu Val Pro Asp Val Glu  
485 490 495  
Ser Arg Glu Asp Leu Ile Lys Asn Arg Tyr Met Ala Arg Ile Val Glu  
500 505 510  
Leu Thr Ser Glu Leu Glu Leu Ala Ser Lys Ser Val His Phe Tyr  
515 520 525  
Ala Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser  
530 535 540  
Lys Glu Ala Leu Thr Glu Glu Met Lys Leu Ala Ser Glu Asn Ile Ser  
545 550 555 560  
Arg Leu Glu Asp Glu Leu Thr Thr Lys Arg Ser Tyr Glu Asp Glu  
565 570 575  
Leu Ser Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser  
580 585 590  
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69

(36)

70

【0204】

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gcctgggcgg cctggcctgt acgggcggg ggaGgc atg gcc tcg ggt ggt ttg 175  
Met Ala Ser Ala Glu Leu  
Met Ala Ser Ala Glu Leu  
1 5  
cag ggg aag tac cag ang ctg gct cag gag tac tcg aag ctt cgg gct 223  
Glu Gly Lys Tyr Glu Lys Leu Ala Glu Glu Tyr Ser Lys Leu Arg Ala  
10 15 20  
cag aat cag gtt ctg aaa aag ggt gtt gTg gat gaa caa gaa aat tct 271  
Glu Asn Glu Val Leu Lys Lys Gly Val Val Asp Glu Glu Ala Asn Ser  
25 30 35  
gaa gct tta aag gag caa ctg aaa atg aag gat cag tca ttg aga aaa 319  
Ala Ala Leu Lys Glu Glu Lys Met Lys Asp Glu Ser Leu Arg Lys  
40 45 50  
cta caa cag gaa atg gac agt ttg aca ttt cga aat ctg cag ctt gcc 367  
Leu Glu Glu Met Asp Ser Leu Thr Phe Arg Asn Leu Glu Leu Ala  
55 60 65 70  
aag agg gta gaa cta ctt caa gat gaa cta gct cta agt gaa cca cga 415  
Lys Arg Val Glu Leu Leu Glu Asp Glu Leu Ala Leu Ser Glu Pro Arg  
75 80 85  
ggc aag aaa aac aag aaa agt gga gaa tct tct cag ttg agt caa 463  
Gly Lys Lys Asn Lys Lys Ser Gly Glu Ser Ser Glu Leu Ser Glu  
90 95 100  
gag cag aag agt gtc ttt gat gaa gat ctg caa aag aag ata gaa gag 511  
Glu Glu Lys Ser Val Phe Asp Glu Asp Leu Glu Lys Lys Ile Glu Glu  
105 110 115  
aet gaa cgg ttg cat ata caa ttt ttt gaa gct gat gag cag cnc aag 559  
Asn Glu Arg Leu His Ile Glu Phe Phe Glu Ala Asp Glu Glu His Lys  
120 125 130  
cat gTg gaa gca gag ctg agg agt cga ctg gcc act ctg gag aca gaa 607  
His Val Glu Ala Glu Leu Arg Ser Arg Leu Ala Thr Leu Glu Thr Glu  
135 140 145 150  
gaa gcc cag cnc caa gct gTg gtt gac ggt ctc acc cgg aag tac atg 655  
Ala Ala Glu His Glu Ala Val Val Asp Gly Leu Thr Arg Lys Tyr Met  
155 160 165  
gaa acc att gag aag ctg cag aac gac aag gct aaa cta gaa gTg aaa 703  
Glu Thr Ile Glu Lys Leu Glu Asn Asp Lys Ala Lys Leu Glu Val Lys  
170 175 180  
tct cag act cta gaa aag gaa gcc aag gaa tgt cga ctt cga aag gaa 751

Ser Glu Thr Leu Glu Lys Glu Ala Lys Glu Cys Arg Leu Arg Thr Glu  
185 190 195 799  
gaa tgt aca tta aag act ctt cat gaa gat ttg tca agt aga  
Glu Cys Glu Glu Lys Thr Leu His Glu Asp Leu Ser Gly Arg  
200 205 210  
tta gag gaa tcc tta tca atc aac aat gaa aaa gta cct ttt aat gat  
Leu Glu Ser Leu Ser Ile Ile Asn Glu Lys Val Pro Phe Asn Asp  
215 220 225 847  
aca aaa tat agt cag tac aac gct ctg aac gtt cca ctc cac aat agg  
Thr Lys Tyr Ser Glu Tyr Asn Ala Leu Asn Val Pro Leu His Asn Arg  
235 240 245  
aga cac cag ctg aag atg cga gat att get ggg cag gcc ctg gct ttt  
Arg His Glu Lys Lys Met Arg Asp Ile Ala Gly Glu Ala Leu Ala Phe  
250 255 260 943  
ggt cag gat ctt gtt aag gct ctt cta aac ttt cat acc tac aca gaa  
Val Glu Asp Leu Val Thr Ala Leu Leu Asn Phe His Thr Tyr Thr Glu  
265 270 275 991  
cag agg att caa att ttt cct gtt gat tct gcc att gac act ata tct  
Gln Arg Ile Glu Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile Ser  
280 285 290  
cca ttt aat cag aag ttc tca aca tcc ctt cat gaa aat gcg tcc tat  
Pro Leu Asn Glu Lys Phe Ser Glu Tyr Leu His Glu Asn Ala Ser Tyr  
295 300 305 1039  
gtc cgc cct ctt gag gaa gga atg ctt cat tta ttt gaa agt atc act  
Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile Thr  
315 320 325  
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Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Thr Phe  
330 335 340 1183  
tca gaa cac tta acc tcc tac ata tgt ttt ctt agg aag att ctt ccc  
Ser Glu His Leu Thr Ser Tyr Ile Cys Phe Leu Arg Lys Ile Leu Pro  
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Tyr Glu Lys Ser Leu Glu Glu Cys Glu Ser Ser Leu Cys Thr  
360 365 370 1279  
tct gcg tta aga gcc agg aat cta gag ctg tcc cag gag atg aaa aaa  
Ser Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Glu Asp Met Lys Lys  
375 380 385 1327  
atg aca gct gtg ttt gag aag ctg cag act tac ata gct ctt ctt gcc  
Met Thr Ala Val Phe Glu Lys Leu Glu Thr Tyr Ile Ala Leu Leu Ala  
395 400 405 1375  
ttg cca agt aca gag cca gat gga ctc ctt cgg aca aac tac agt tct  
Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Ser Ser  
410 415 420 1423  
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Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val Met  
425 430 435 1471  
aaa gat att tcc aaa cat tat agt caa aaa gct gca ata gag cat gaa  
Lys Asp Ile Ser Lys His Tyr Ser Glu Lys Ala Ala Ile Glu His Glu  
440 445 445 1519

440 445 450 1567  
ctt cca aca gca aca cag aag ctg ata aca act aat gac tgt atc ctg  
Leu Pro Thr Ala Thr Glu Lys Leu Ile Thr Thr Asn Asp Cys Ile Leu  
455 460 465 470  
tca tca gta gtg gca tta aca aat gga gca gga aag att gca tcc ttc  
Ser Ser Val Val Ala Leu Thr Asn Gly Ala Gly Lys Ile Ala Ser Phe  
475 480 485 1615  
ttc agc aac aat ttg gac tac ttc att gct tca ctg agc tat gga cct  
Phe Ser Asn Asn Leu Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly Pro  
490 495 500 1663  
aag gca gcg agt gga ttc att agt cct ctt tca gct gaa tgc atg cta  
Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met Leu  
505 510 515 1711  
cag tat aag aaa aat get gcc tat atg aag tct ttg aga aag ccc  
Gln Tyr Lys Lys Lys Ala Ala Tyr Met Lys Ser Leu Arg Lys Pro  
520 525 530 1759  
ctc ttg gag tct gtg cct tat gaa gaa gca ctg gca aac cgc cgc atc  
Leu Leu Glu Ser Val Pro Tyr Glu Glu Ala Leu Ala Asn Arg Arg Ile  
535 540 545 550 1807  
ctt ctc agc tct act gaa agt cga gaa ggc ctt gca cag caa gtt caa  
Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Glu Glu Val Glu  
555 560 565 1855  
cag agt ttg gaa aag att tct aaa ctg gag cag gaa aaa gaa cat tgg  
Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu Glu Glu Lys Glu His Trp  
570 575 580 1903  
atg ttg gaa gca caa tta gcc aaa atc aag cta gag aaa gaa aac cag  
Met Leu Glu Ala Glu Leu Ala Lys Ile Lys Leu Glu Lys Glu Asn Glu  
585 590 595 1951  
cga att gca gat aag ctg aag aat aca ggt agt gcc cag ctg gtt ggg  
Arg Ile Ala Asp Lys Leu Lys Asn Thr Cys Ser Ala Glu Leu Val Gly  
600 605 610 1999  
ctg gcc cag gaa aat gct gct gtg tca aat act gct ggc cag gat gaa  
Leu Ala Glu Glu Asn Ala Ala Val Ser Asn Thr Ala Gly Glu Asp Glu  
615 620 625 2047  
gcc aca gct aag gct gtg ttg gag ccc att cag agc acc agt cta att  
Ala Thr Ala Lys Ala Val Leu Glu Pro Ile Glu Ser Thr Ser Leu Ile  
635 640 645 2143  
ggg act tta acc agg aca tct gac agt gag gtt cca gat gtg gaa tct  
Gly Thr Leu Thr Arg Thr Ser Asp Ser Glu Val Pro Asp Val Glu Ser  
650 655 660 2191  
cgt gaa gac tta att aaa aat cac tac atg gca agg ata gtg gaa ctt  
Arg Glu Asp Leu Ile Lys Asn His Tyr Met Ala Arg Ile Val Glu Leu  
665 670 675 2239  
acg tct cag ttg cag ctg gct gac agt aag tca gtg cat ttt tat gcc  
Thr Ser Glu Leu Glu Leu Ala Asp Ser Lys Ser Val His Phe Tyr Ala  
680 685 690 2287  
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Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser Lys  
695 700 705 710

75

76

gaa gca ttg aca gaa atg aaa ctt gcc agt cag aac atc agc aga 2335  
 Glu Ala Thr Thr Glu Glu Met Lys Leu Ala Ser Glu Asn Ile Ser Arg 725  
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 ctt cag gat gag ctg aca act acc aag agg agt tac gag gat cag tta 2383  
 Leu Glu Asp Glu Leu Thr Thr Lys Arg Ser Tyr Glu Asp Glu Leu 740  
 730 735  
 agt atg atg agt cag cac ctg tgc agc atg aat gag aca tta tct aaa 2431  
 Ser Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser Lys  
 745 750 755  
 cag aga gaa gag att gac aca cta aag atg tcc agt aag ggg aat tct 2479  
 Glu Arg Glu Glu Ile Asp Thr Thr Leu Lys Met Ser Ser Lys Glu Asn Ser 760  
 765 770  
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[0205]

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 Ala Ser Ala Ala Ser Pro Ala Val Ala Leu Lys Ala Leu Glu Val Glu 5  
 10 15  
 att gtt gag gag ggc act cag aac ggc gag gag cag cag agt act ttc 153  
 Ile Val Glu Glu Ala Thr Glu Asn Ala Glu Glu Glu Glu Pro Ser Thr Phe 20  
 25 30 35  
 agc gaa aat gag tat gat gcc agt tgg tcc cca tca tgg gtc atg tgg 201  
 Ser Glu Asn Glu Tyr Asp Ala Ser Trp Ser Pro Ser Trp Val Met Trp 40  
 45 50  
 ctt ggg ctt ccc agc aca ctt cat agc tgc cac gat ata gtt tta cga 249

77

78

Leu Gly Leu Pro Ser Thr Leu His Ser Cys His Asp Ile Val Leu Arg 65  
 55 60  
 aga agt tac ttg gga agt tgg ggc ttt agt atc gtt ggt gga tat gaa 297  
 Arg Ser Tyr Leu Gly Ser Trp Gly Phe Ser Ile Val Gly Gly Tyr Glu 80  
 70 75  
 gag aac cac acc aat cag cct ttt ttc att aaa act att gtc ttg gga 345  
 Glu Asn His Thr Asn Glu Pro Phe Phe Ile Lys Thr Ile Val Leu Gly 95  
 85 90  
 act cct gct tat tat gat gga aga tta aag tgt ggt gac atg att gtc 393  
 Thr Pro Ala Tyr Tyr Asp Gly Arg Leu Lys Cys Gly Asp Met Ile Val 110  
 100 105 115  
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 Ala Val Asn Gly Leu Ser Thr Val Gly Met Ser His Ser Ala Leu Val 120  
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 Pro Met Leu Lys Glu Glu Arg Asn Lys Val Thr Leu Thr Val Ile Cys 135  
 140 145  
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 Trp Pro Gly Ser Leu Val 150  
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[0206]

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 Cys Leu Ser Arg Phe Leu Gly Trp Phe Arg Gln Pro Val Leu Val 25  
 15 20  
 act cag tcc gca gct ata gtt cca gta aga act aaa aaa cgt ttc aca 147  
 Thr Gln Ser Ala Ala Ile Val Pro Val Arg Thr Lys Lys Arg Phe Thr 35  
 30 40  
 cct cct att tat caa cct aaa ttt aaa aca gaa aag gag ttt atg caa 195  
 Pro Pro Ile Tyr Gln Pro Lys Phe Lys Thr Glu Lys Glu Phe Met Gln 50  
 45 55  
 cat gcc cgg aaa gaa gga ttg gtt att cct cca gaa aaa tgc gac cgt 243  
 His Ala Arg Lys Ala Gly Leu Val Ile Pro Pro Glu Lys Ser Asp Arg 65  
 70  
 tcc ata cat ctg gcc tgt aca gct ggt ata ttt gat gcc tat gtt cct 291  
 Ser Ile His Leu Ala Cys Thr Ala Gly Ile Phe Asp Ala Tyr Val Pro 80  
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 cct ggc ggt gat gca cgc ata tca tct ctt tca aag gag gga ctg ata 339  
 Pro Glu Gly Asp Ala Arg Ile Ser Ser Leu Ser Lys Glu Gly Leu Ile 95  
 100  
 gag aga act gaa cga atg aag aag act atg gca tca cca gfg tca atc 387  
 Glu Arg Thr Arg Arg Met Lys Lys Thr Met Ala Ser Gln Val Ser Ile 110  
 115 120  
 cgg agg ata aaa gac tat gat gcc aac ttt aaa ata aag gac ttc cct 435  
 Arg Arg Ile Lys Asp Tyr Asp Ala Asn Phe Lys Ile Lys Asp Phe Pro 125  
 130 135  
 gga aaa gct aag gat atc ttt att gaa gct cac ctt tgt cta aat aac 483  
 Gly Lys Ala Lys Asp Ile Phe Ile Glu Ala His Leu Cys Leu Asn 140  
 145 150  
 tca gac cat gac cga ctt cat acc ttg gta act gaa cac tgt ttt cca 531  
 Ser Asp His Asp Arg Arg Leu His Thr Leu Val Thr Glu His Cys Phe Pro 155  
 160 165  
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 Asp Met Thr Trp Asp Ile Lys Tyr Lys Thr Val Arg Trp Ser Phe Val 175  
 180 185  
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 Glu Ser Leu Glu Pro Ser His Val Val Gln Val Arg Cys Ser Ser Met 190  
 195 200  
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 210 215  
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 Arg Gln Thr Leu Ala Ile Tyr Asp Arg Phe Gly Arg Leu Met Tyr Gly 220  
 225 230  
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81 82

Gln Glu Asp Val Pro Lys Asp Val Leu Glu Tyr Val Val Phe Glu Lys 235  
 240 245  
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 Gln Leu Thr Asn Pro Tyr Gly Ser Trp Arg Met His Thr Lys Ile Val 255  
 260 265  
 ccc cca tgg gca ccc cct aag cag ccc atc ctt aag aag gfg aag atc 867  
 Pro Pro Trp Ala Pro Pro Lys Gln Pro Ile Leu Lys Thr Val Met Ile 270  
 275 280  
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 Pro Gly Pro Gln Leu Lys Pro Glu Glu Tyr Glu Glu Ala Gln Gly 285  
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 Glu Ala Gln Lys Pro Gln Leu Ala 300  
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【0207】

83

84

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86

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89

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[0209]

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[0210]

88

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Ala Arg Asn Leu Glu Leu Ser Cln Asp Met Lys Lys Met Thr Ala Val  
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Phe Glu Lys Leu Cln Thr Tyr Ile Ala Leu Leu Ala Leu Pro Ser Thr  
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【0221】

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【図面の簡単な説明】

【図1】は、PCR法を用いて、35種のヒト組織（臓器）におけるCOL03279転写物の発現量を調べた結果である。

【図2】は、PCR法を用いて、35種のヒト組織（臓器）におけるCOL06772転写物の発現量を調べた結果である。

【図3】は、PCR法を用いて、35種のヒト組織（臓器）におけるADKA01604転写物の発現量を調べた結果である。

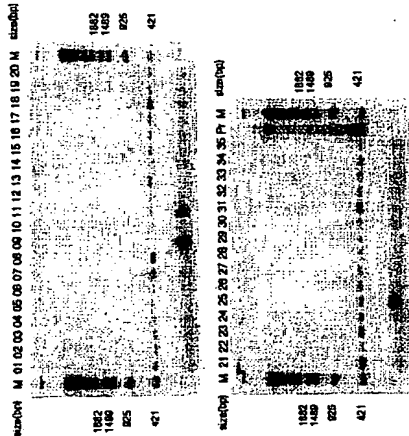
【図4】は、PCR法を用いて、35種のヒト組織（臓器）におけるADSU00701転写物の発現量を調べた結果である。

【符号の説明】

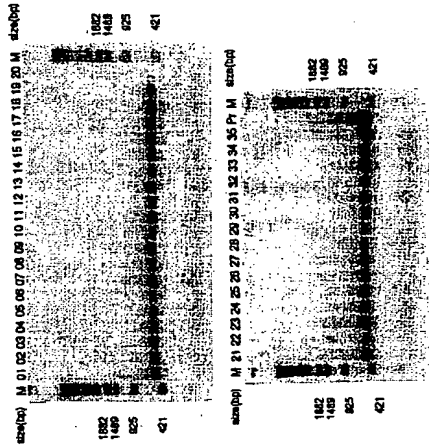
全図中に記載の数字、英字は以下の通りである。

01：脳、02：脳、03：風状核、04：海馬、05：黒質、06：視床、07：腎臓、08：脾臓、09：膵下垂体、10：小腸、11：腎臓、12：扁桃腺、13：小腸、14：膵臓、15：胎児肝臓、16：胎児腎臓、17：胎児肝臓、18：胎児脾臓、19：心臓、20：肝臓、21：脾臓、22：リンパ節、23：腎臓、24：胎盤、25：前立腺、26：唾液腺、27：骨格筋、28：腎臓、29：脾臓、30：胃、31：精巣、32：膵臓、33：甲状腺、34：気管、35：子宮、Pr：プラスミド、M：分子重量マーカー

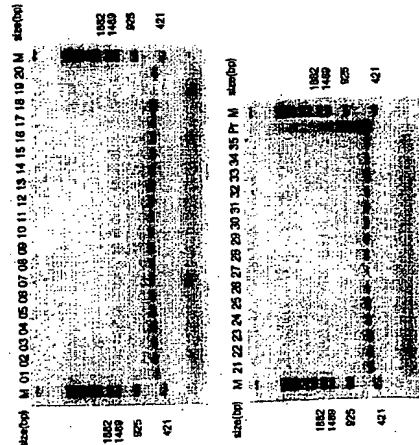
【図2】



【図3】



【図1】





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A61K 39/395  
A61K 48/00  
A61P 3/10  
A61P 9/04  
A61P 9/10  
A61P 11/00  
A61P 11/02  
A61P 11/04  
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A61P 37/04  
A61P 37/08  
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// C12P 21/08  
(C12N 1/21  
C12R 1:19 )  
(C12N 5/10  
C12R 1:91 )

(21)Application number : 2000-175475 (71)Applicant : KYOWA HAKKO KOGYO CO LTD  
(22)Date of filing : 12.06.2000 (72)Inventor : OBATA CHOEI  
NISHI TATSUYA  
OTA NORIO  
NAKAMURA YUSUKE  
SUGANO SUMIO

(54) NEW POLYPEPTIDE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a polypeptide useful for screening for and/or developing an agent for treating, preventing, and/or diagnosing a disease related to the activation of NF-κB, a DNA encoding the polypeptide, an antisense DNA/RNA of the DNA the gene therapy using the DNA, an antibody recognizing the polypeptide, a modified polypeptide derived from the preceding polypeptide and having an enhanced activity, a dominant negative variant of the polypeptide, and methods for utilizing these.  
SOLUTION: A polypeptide activating NF-κB is identified to produce a DNA encoding the polypeptide and an antibody recognizing the polypeptide. These can be utilized for screening for a medicine for and diagnosing a disease related to the activation of NF-κB.

LEGAL STATUS

[Date of request for examination]  
[Date of sending the examiner's decision of rejection]  
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]  
[Date of final disposal for application]  
[Patent number]  
[Date of registration]  
[Number of appeal against examiner's decision of rejection]  
[Date of requesting appeal against examiner's decision of rejection]  
[Date of extinction of right]

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

## CLAIMS

- [Claim(s)]
- [Claim 1] The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5.
- [Claim 2] The polypeptide which has the activity which one or more amino acid consists [activity] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and raises the activity of NF-kappa B.
- [Claim 3] The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.
- [Claim 4] DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.
- [Claim 5] DNA which has the base sequence expressed with either of the array numbers 6-10.
- [Claim 6] DNA which carries out the code of the polypeptide which has the activity which it is [activity] J DNA according to claim 4 or 5 and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.
- [Claim 7] The recombinant vector which includes DNA of a publication in any 1 term of claims 4-6 at a vector, and is obtained.
- [Claim 8] The recombinant vector which includes in a vector RNA which becomes any 1 term of claims 4-6 from DNA of a publication, and a homologous array, and is obtained.
- [Claim 9] The recombinant vector according to claim 8 whose RNA is a single strand.
- [Claim 10] The transformant which holds a recombinant vector according to claim 7.
- [Claim 11] The transformant according to claim 10 whose transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.
- [Claim 12] The transformant according to claim 11 whose microorganism is a microorganism belonging to an Escherichia group.
- [Claim 13] The transformant according to claim 11 whose animal cell is an animal cell chosen from a mouse myeloma cell, a rat myeloma cell, a mouse hybridoma cell, a CHO cell, a BHK cell, an African green monkey kidney cell, a Namalwa cell, a Namalwa KJM-1 cell, a Homo sapiens embryo kidney cell, and a Homo sapiens leukemic cell.
- [Claim 14] The transformant according to claim 11 whose insect cell is an insect cell chosen from the ovarian cell of Spodoptera frugiperda, the ovarian cell of Trichoplusia ni, and the ovarian cell of a silkworm.
- [Claim 15] The transformant according to claim 10 whose transformant is a nonhuman transgenic animal or a transgenic plant.
- [Claim 16] The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of claims 10-14 to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into a culture, and is characterized by extracting this polypeptide from this culture.
- [Claim 17] The manufacture approach of this polypeptide which breeds the nonhuman transgenic

animal which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this animal, and is characterized by extracting this polypeptide from the inside of this animal.

[Claim 18] The manufacturing method according to claim 17 characterized by are recording being among the milk of an animal.

[Claim 19] The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA according to claim 7, is made to generate and accumulate the polypeptide of a publication in any 1 term of claims 1-3 into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.

[Claim 20] The manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in vitro using DNA given in any 1 term of claims 4-6.

[Claim 21] The antibody which recognizes the polypeptide of a publication in any 1 term of claims 1-3.

[Claim 22] The oligonucleotide or this nucleotide which has the array which consists of five to 60 base by which any 1 term of claims 4-6 was followed in the base sequence of DNA of a publication, and the oligonucleotide which has a complementary array.

[Claim 23] How to detect the manifestation including carrying out hybridization to any 1 term of claims 4-6, using DNA or the oligonucleotide according to claim 22 of a publication as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.

[Claim 24] How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide according to claim 22 as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.

[Claim 25] How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of claims 1-3 by the hybridization method using DNA or the oligonucleotide according to claim 22 of a publication in any 1 term of claims 4-6.

[Claim 26] How to detect the variation including performing polymerase chain reaction using an oligonucleotide according to claim 22 of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.

[Claim 27] An approach given in any 1 term of claims 23-26 used in order to detect the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.

[Claim 28] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach according to claim 27 the disease accompanied by activation of unusual immunocyte is allergy atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.

[Claim 29] How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6, or the translation of mRNA.

[Claim 30] How to acquire the promoter region and the imprint regulatory region of DNA which

are characterized by using DNA or the oligonucleotide according to claim 22 of a publication for any 1 term of claims 4-6 and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3.

[Claim 31] Physic which contains the polypeptide of a publication in any 1 term of claims 1-3.

[Claim 32] Physic which contains the recombinant vector of a publication in DNA given in any 1 term of claims 4-6, claim 8, or any 1 term of 9.

[Claim 33] Physic containing an antibody according to claim 21.

[Claim 34] Physic containing an oligonucleotide according to claim 22.

[Claim 35] Physic according to claim 31 characterized by a polypeptide having an immunity activation operation.

[Claim 36] Physic according to claim 35 characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.

[Claim 37] Physic given in any 1 term of claims 32-34 whose physic is the physic for the therapy of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[Claim 38] Physic given in any 1 term of claims 32-34 whose physic is the physic for a diagnosis of the disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by the failure of a pancreas cell, the disease accompanied by activation of an unusual osteoclast, the disease accompanied by activation of unusual immunocyte, or the disease accompanied by unusual cell proliferation.

[Claim 39] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, Physic according to claim 37 or 38 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[Claim 40] It is characterized by using the polypeptide of a publication for any 1 term of claims 1-3. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[Claim 41] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent

diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening procedure according to claim 40 whose disease accompanied by unusual cell proliferation it is pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[Claim 42] Physic which acts on a polypeptide given in any 1 term of claims 1-3 acquired by the screening approach according to claim 40 or 41 specifically.

[Claim 43] It is characterized by using the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 obtained by the approach according to claim 30. The disease accompanied by infection or inflammation, the disease accompanied by differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by activation of an unusual osteoclast, The medicinal screening approach for the therapy of the disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[Claim 44] The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy, asthma, The medicinal screening approach according to claim 43 that it is pollinosis, respiratory tract irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a nerve cell is an Alzheimer disease or ischemic encephalopathy.

[Claim 45] Physic which acts on the promoterregion and the imprint regulatory region of DNA which are obtained by the screening approach according to claim 43 or 44, and which carry out the code of the polypeptide of a publication to any 1 term of claims 1-3 specifically.

[Claim 46] The immunological detecting method of a polypeptide given in any 1 term of claims 1-3 characterized by using an antibody according to claim 21.

[Claim 47] The immunity staining method characterized by detecting the polypeptide of a publication in any 1 term of claims 1-3 using an antibody according to claim 21.

[Claim 48] How to screen the matter which controls or promotes the imprint or translation of DNA which is characterized by using an antibody according to claim 21, and which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3.

[Claim 49] The manifestation of DNA which carries out the code of the polypeptide of a publication to any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.

[Claim 50] The activity which the polypeptide of a publication has in any 1 term of claims 1-3 is a part or the knock out nonhuman animal controlled completely.

[Claim 51] The screening approach of a variant polypeptide characterized by using the



polypeptide of a publication for any 1 term of claims 1-3 of having dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.

[Claim 52] The variant polypeptide which is obtained by the screening approach according to claim 51 and which has dominant negative activity to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.

[Claim 53] DNA which carries out the code of the variant polypeptide according to claim 52.

[Claim 54] The screening approach of a variant polypeptide characterized by using the polypeptide of a publication for any 1 term of claims 1-3 of having the variation which raises this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of claims 1-3.

[Claim 55] The variant polypeptide which is acquired by the screening approach according to claim 54 and to which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term of claims 1-3.

[Claim 56] DNA which carries out the code of the variant polypeptide according to claim 55.

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[Translation done.]

## \* NOTICES \*

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
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## DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] DNA which carries out the code of a polypeptide with new this invention, and this polypeptide. The transformant which holds the recombinant DNA which includes this DNA in a vector and is obtained, and this recombinant DNA. The manufacturing method of this polypeptide using this transformant, the analysis method of the amount of manifestations of this DNA and variation which used the oligonucleotide obtained from this DNA. The immunity staining method using the antibody and this antibody which recognize this polypeptide, the activity rise alteration object which introduced variation into this polypeptide by deletion, insertion, a permutation, etc., The dominant negative variant which introduced variation into this polypeptide by deletion, insertion, etc., The screening procedure of a compound which fluctuates the activity of this polypeptide, the screening procedure of a compound which fluctuates the manifestation of this DNA. It is related with the compound obtained by the screening procedures of a compound which fluctuate the effectiveness of the imprint by the promoter DNA who manages the imprint of this DNA, and this promoter DNA, and these screening procedures, the knock out animal to which this DNA was suffered a loss or mutated.

[0002]

[Description of the Prior Art] nuclear factor-kappaB (following, NF-kappaB) was identified as a transcription factor to be combined with the enhancer in connection with the immunoglobulin light chain (Ig light chain) gene expression in a B cell in 1986 [Cell, 46, 705-716 (1986), Cell, and 47,921-928 (1986)].

[0003] NF-kappa B consists of heterodimers of two or more molecules belonging to a Rel family, and NF-kappa B generally guided in many cells is considered to be the heterodimer of p50 and RelA [Mol.Cell.Biol., 12, and 674-684 (1992)]. Existence of the factor IkappaB which controls NF-kappa B has also become clear. IkappaB by forming NF-kappa B and complex at the time of no stimulating, and carrying out the mask of the nuclear shift signal of NF-kappa B [Science which has controlled nuclear shift, 242, and 540-546 (1988), Cell, 65, 1281-1289 (1991), Cell, 68, and 1109-1120 (1992), EMBO J., 12, 3893-3901 (1993), Cell, 78, 773-785 (1994), Cell, 87, and 13-20 (1996) ---]. The signal transfer molecule which IkappaB will mention later if a cell is stimulated by a tumor necrosis factor alpha (following, TNF-alpha) etc. --- 32 and the 36th serine --- phosphorylation --- it continues, and it is ubiquitin-ized and is decomposed by proteasome. If IkappaB is decomposed, the shift to a nucleus of NF-kappa B will be attained, and it will come to guide various gene expression with an enhancer [Cell, 80, 529-532 (1995), Cell, 80, and 57 3-582 (1995)].

[0004] As the matter which activates NF-kappa B, or a stimulus, cytokine [TNF-alpha, A tumor necrosis factor beta (following, TNF-beta), interleukin 1 alpha (Following and IL-1alpha), interleukin 1 beta (following and IL-1beta) ], such as interleukin 2 (the following, IL-2) and a leukemia inhibitor (following, LIF), T cell mitogen (an antigen stimulus, lectin, and an anti-T cell receptor antibody ---) Anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, B cell mitogen (an anti-IgM antibody, anti-CD40), leukotriene, Lipopolysaccharide (following, LPS), phorbol myristate acetate (Following, PMA), parasitism somesthesis stain, and

virus infection [human immunodeficiency virus (The following, HIV-1), a human T cell leukemia virus 1 (the following, HTLV-1), A hepatitis B virus (following, HBV), an Epstein-Barr virus (The following, EBV), a cytomegalovirus (following, CMV), a herpes simplex virus 1 (The following, HSV-1), a human herpesvirus 6 (the following, HHV-6) ], such as Newcastle disease virus (following, NDV), Sendai Virus, and adenovirus, A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) Ultraviolet rays, a radiation, oxidation stress, etc. are known [Biochemica et Biophysica Acta, 1072, 63-80 (1991), Annu.Rev.Cell Biol.10, and 405-455 (1994)].

[0005] moreover, as a molecule in which an induction manifestation is carried out by activation of NF-kappa B (1) To an inflammatory response and an immune response at control of a \*\*\* molecule group and (2) apoptosis \*\*\*\* molecule group, (3) The \*\*\* molecule group, the molecule group about (4) viruses, etc. are known by generating and differentiation. [Biochemica et Biophysica Acta, 1072, and 63-80 (199 1), Annu.Rev.Cell Biol.10, 405-455 (1994)], and an induction manifestation are various.

[0006] As a molecule by which an induction manifestation is carried out, specifically Cytokine [IL-1alpha, IL-1beta, IL-2, interleukin 3 (the following, IL-3), interleukin 6 (The following, IL-6), interleukin 8 (the following, IL-8), interleukin 12 (The following, IL-12), TNF-alpha, TNF-beta, interferon beta ], a cell growth factor [macrophage colony-stimulating factor (The following, IFN-beta) (The following, M-CSF), a granulocyte macrophage colony-stimulating factor (Following and GM-CSF), granulocyte colony-stimulating factor (following, G-CSF)], A receptor [interleukin 1 receptor (following and IL-1R) antagonist, The interleukin 2 receptor alpha (following and IL-2Ralpha), an immunoglobulin kappa light chain (The following, Ig-kappa-LC), T-cell receptorbeta, a major histocompatibility antigen Classes 1 and II, beta 2-microglobulin], adhesion factor [endothelialleucocyte adhesionmolecule-1 (The following, MHC) (The following, ELAM-1), vascula r cell adhesionmolecule-1 (Following and VCAM-1) intercellularadhesion molecule-1 (The following, ICAM-1) ] and acute stage protein (blood serum amyloid A precursor protein ---) Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, An induction type NO synthase (following, iNOS), cyclooxygenase 2 (The following, COX-2), a vascular endothelial cell growth factor acceptor (following, VEGF-R2), Transcription factor [c- rel, p105, I kappa-alpha, c-Myc, an interferon regulator ], vimentin, virus [HIV-1, HIV-2, a rhesus monkey immunodeficiency disease virus (The following, IRF-1) (The following, SIMmac), CMV, HSV-1, the rhesus monkey virus 40 (following, SV40), adenovirus], etc. are known [a protein nucleic-acid enzyme, 41, and 1198-1209 (1996)].

[0007] As for the signal transfer about NF-kappa B activation, the elucidation is progressing about TNF-alpha and IL-1. In the activation signal from TNF-alpha A TNF receptor (TNFR1 or TNFR2), TNF receptor-associate d death domain protein (The following, TRADD), TNFR-associated factor -2 (The following, TRAF2), receptor interacting protein (The following, RIP), NF-kappa B-inducing kinase (The following, NIK), IkappaB kinase (following, IKK) [IKKalpha, IKKbeta, IKKgamma (NEMO)], IKK-co mplex-associated protein (following, IKAP), etc. are found out as an activation molecule. [EMBO J., 14, and 2876-288 3 (1995), Science, 267, and 1485-1489 (1995), GENES & DEVELOPMENT, 9, 1586-1597 (1995), Cell, 84, 853-862 (1996), Nature, 388, and 548-554 (1997), Cell, 90,373-383 (1997), Science, 278, and 860-866 (1 997), Science, 278, 866-869 (1997), Cell, 91, 243-252 (1997), Nature, 395, and 292-296 (1998) ---].

[0008] In the activation signal from IL-1 IL-1 receptor 1 (Following and IL-1R) IL-1 receptor accessory protein (Following and IL-1RAcP), Myd88, IL-1 receptor-associated kinase TNF receptor-associated factor 6 (The following, IRAK) (The following, TRAF6) and TAK1 binding protein 1 [Science by which (the following, TAB1), Transforming gro wth factor-beta-activated kinase 1 (TAK1), etc. are found out as an activation molecule, 270, and 2008-2011 (1995), Nature, 398, 252-256 (1999)].

[0009] It has been thought that the enzyme (NF-kappa B kinase) which phosphorizes NF-kappa B is concerned with enhancement of a NF-kappa B signal on the other hand [J.Biol.Chem.268, 26790-26795 (1993), EMBO J.13, and 4597-4607 (1994)]. As mentioned above, although it is known that very many molecules are participating in activation of NF-kappa B, all the role of the

identified molecules is not solved. In the stimulus of those other than TNF- $\alpha$ , such as ultraviolet rays and oxidation stress, or IL-1, the actual condition is that most molecules in connection with activation of NF- $\kappa$ B are not solved, furthermore -- even if it sees the tissue specific expression of a Rel family molecule -- an organization -- [Science, 284, 313-316 (1999), Science, 284, 316-320 (1999), Science, 284, 321-325 (1999), Immunity, 10, 421-429 (1999), Nature Genet, 22, and 74-77] the activation device of specific NF- $\kappa$ B is expected to be (1999).

[0010] As mentioned above, it is very useful it to be thought for that many [ still ] strange molecules in the living body concerned with activation of NF- $\kappa$ B exist, and to discover and use these genes for the therapy of the disease in which an elucidation or NF- $\kappa$ B of symptoms participates. NF- $\kappa$ B is bearing the very important role in rise of an immune response in the living body so that the molecule group which carries out an induction manifestation by activation of the molecule group which activates NF- $\kappa$ B mentioned above, or NF- $\kappa$ B may also show. The cytokine of TNF- $\alpha$  which has antitumor or antiviral activity, or IL-1 grade demonstrates a part for the principal part of the operation through activation of NF- $\kappa$ B. Moreover, the cytokine which carries out an induction manifestation by NF- $\kappa$ B, such as IL-1, IL-2, IL-12, TNF- $\alpha$ , and IFN- $\beta$ , also rises the immunoreaction in a living body or an organization, and has antitumor or antiviral activity.

[0011] Thus, it is a well-known fact that activation of NF- $\kappa$ B controls a neoplasm and a virus in an actual disease, and it is thought that the thing of in the living body or a living body for which the activity of NF- $\kappa$ B is artificially raised in an organization in part is very effective in rise of an immune response or enhancement of antitumor and antiviral activity. Therefore, discovery and acquisition of a NF- $\kappa$ B activation rise variant are still very more useful in discovery of DNA which carries out the code of the polypeptide and it which activate NF- $\kappa$ B and acquisition, and the physic that used antitumor and antiviral one as the target.

[0012] On the other hand, cytokine, such as IL-1 which carries out an induction manifestation by NF- $\kappa$ B, IL-6, IL-8, and TNF- $\alpha$ , is also called inflammatory cytokine, and the immune response which rose too much by these cytokine causes various diseases. These cytokine activates a macrophage, neutrophil leucocyte, a lymphocyte, etc., and works towards exacerbation in an inflammatory tissue. Moreover, the adhesion molecules of ELAM-1, VCAM-1, and ICAM-1 grade guided by NF- $\kappa$ B [Mol.Cell.Biol. which promotes infiltration in the organization of a leucocyte and rises accumulation of the leucocyte in an inflammatory tissue, 14, and 5701 (1994), Mol.Cell.Biol., 14, 5820 (1994), Pro.Nat.Acad.Sci USA, 90, and 3943 (1993) - ]. The enzyme of iNOS or COX-2 grade produces a nitrogen monoxide (following, NO) and prostaglandin E2, respectively, and acts on the escape of acute inflammation or a blood vessel. [0013] That is, it is thought that NF- $\kappa$ B is bearing the central role in acute inflammation and the chronic inflammation through these cells or molecules. Activation of NF- $\kappa$ B is actually reported by the synovial membrane of rheumatoid arthritis, the intestinal tract of Crohn's disease, and asthmatic lung tissue. Therefore, in the disease at large in which inflammation, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, chronic hepatitis B, chronic hepatitis C, graft versus host disease, an insulin dependency and non-dependency diabetes mellitus, traumatic brain injury, inflammatory bowel disease, septicemia, and microorganism infection, participates, NF- $\kappa$ B is the important target of a symptoms elucidation and remedy development.

[0014] In connection with cancer, EBV is considered for a Burkitt lymphoma (Burkitt lymphoma), the Hodgkin (Hodgkin) disease, T and B, a spontaneous killer cell lymphoma, EBV related gastric cancer, etc. as a cause. TRADD, TRAF, and association are possible for latent membrane protein (the following, LMP1) in which especially EBV carries out a code, a host's NF- $\kappa$ B is activated, and it is thought that it is participating in immortalization [EMBO J., 16, 6478-6485 (1997), J.Virology, 69, 2168-2174 (1995), Oncogene, 18, 7161-7167 (1999), Gene Th erapy, and 5,905-912 (1998)]. Moreover, adult T-cell leukemia (adult T-cell leukemia: ATL) Tax infection by HTLV-1 is the cause and especially HTLV-1 carries out [ Tax ] a code NF- $\kappa$ B is activated through association to IkappaB, or activation of IKK. It is thought that apoptosis is checked [J.Biol.Chem., 273, 15891-15894 (1999), J.Biol.Chem., 274, and 34417-34424 (1999)]. The various

adhesion molecules which NF- $\kappa$ B guides are participating in transition of a cancer cell, and the vascularization through the apoptosis inhibition activity and VEGF-R2 by NF- $\kappa$ B is participating in growth of a cancer cell. As mentioned above, NF- $\kappa$ B is an important innovative drug development or a therapy target also in the field of cancer.

[0015] Furthermore, also in the viral disease which contains NF- $\kappa$ B other than cancers, such as an acquired immunodeficiency syndrome, as a transcription factor, NF- $\kappa$ B is an important innovative drug development or a therapy target. Moreover, there is a report called a cause and control of the cellular infiltration also according [ ischemia re-reflux failures, such as ischemic encephalopathy, ] to NF- $\kappa$ B activation and apoptosis etc. is considered that NF- $\kappa$ B has played the important role in the onset of the disease accompanied by unusual differentiation growth of a smooth muscle cell including arteriosclerosis, the restenosis, etc. [0016] Although it has been shown clearly that it is what the anti-inflammatory activity of a steroid, the anti-inflammatory activity of aspirin, etc. depend on inhibition of NF- $\kappa$ B, there are no drugs screened as what checks specifically [Science, 270, 283-286 (1995), Science, 270, 286-290 (1995), Molecular and Cellular Biology, 15 and 943-953 (1995)] and NF- $\kappa$ B in recent years. It also has many troubles that the drugs known as a thing in connection with inhibition of the existing NF- $\kappa$ B have that a side effect is strong, and low selectivity and singularity etc., and compound retrieval to which NF- $\kappa$ B was targeted for the purpose of development of a powerful and new antiinflammatory drug with few side effects is performed. As mentioned above, the new polypeptide which activates NF- $\kappa$ B is useful on industry, and acquisition of DNA which carries out the code of these polypeptides and it has been called for. [0017]

[Problem(s) to be Solved by the Invention] This invention Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury, The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Remedies, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), DNA which carries out the code of a useful polypeptide and this polypeptide to retrieval of a prophylactic and a diagnostic drug and development, It is in offering the antibody which recognizes the gene therapy using the antisense DNA/RNA of this DNA, and this DNA, and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions.

[0018]

[Means for Solving the Problem] As a result of inquiring wholeheartedly in order to solve the above-mentioned technical problem, this invention persons succeed in acquiring DNA which carries out the code of the factor to which activation of NF- $\kappa$ B including a new amino acid sequence is urged, and this factor, and came to complete this invention. That is, this invention relates to the following (1) - (54).

[0019] (1) The polypeptide which has the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5. (2) The polypeptide which has the activity which one or more amino acid consists [ activity ] of deletion and amino acid sequences permuted and/or added in the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array

numbers 1-5, and raises the activity of NF-kappa B.

[0020] (3) The polypeptide which has the activity which raises the activity of NF-kappa B, including the amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the array numbers 1-5, and the amino acid sequence which has 60% or more of homology.

(4) (1) DNA which carries out the code of the polypeptide of a publication to any 1 term of - (3).

(5) DNA which has the base sequence expressed with either of the array numbers 6-10.

[0021] (6) DNA which carries out the code of the polypeptide which has the activity which it is [ activity ] DNA given in (4) or (5), and DNA hybridized under stringent conditions, and raises the activity of transcription factor NF-kappa B.

(7) (4) Recombinant vector which includes DNA of a publication in any 1 term of - (6) at a vector, and is obtained.

(8) (4) Recombinant vector which includes in a vector RNA which becomes any 1 term of - (6) from DNA of a publication, and a homologous array, and is obtained.

[0022] (9) The recombinant vector given in (8) given RNA is a single strand.

(10) The transformant which holds a recombinant vector given in (7).

(11) The transformant given in (10) a given transformant is a transformant chosen from the group which consists of a microorganism, an animal cell, a plant cell, and an insect cell.

(12) The transformant given in (11) a given microorganism is a microorganism belonging to an Escherichia group.

[0023] (13) an animal cell -- a mouse -- myeloma -- a cell -- a rat -- a myeloma -- a cell -- a mouse -- a hybridoma -- a cell -- CHO -- a cell -- BHK -- a cell -- an African green monkey -- the kidney -- a cell -- Namalwa KJM -- one -- a cell -- Homo sapiens -- an embryo -- the kidney -- a cell -- and -- Homo sapiens -- a leukemic cell -- from -- choosing -- having -- an animal cell -- it is -- ( -- 11 -- ) -- a publication -- a transformant .

(14) The transformant given in (11) a given insect cell is an insect cell chosen from the ovarian cell of *Spodoptera frugiperda*, the ovarian cell of *Trichoplusia ni*, and the ovarian cell of a silkworm.

[0024] (15) The transformant given in (10) a given transformant is a nonhuman transgenic animal or a transgenic plant.

(16) (10) The manufacture approach of this polypeptide which cultivates a transformant given in any 1 term of - (14) to a culture medium, is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into a culture, and is characterized by extracting this polypeptide from this culture.

[0025] (17) The manufacture approach of this polypeptide which breeds the nonhuman transgenic animal which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this animal, and is characterized by extracting this polypeptide from the inside of this animal.

(18) The manufacturing method given in (17) characterized by are recording being among the milk of an animal.

[0026] (19) The manufacturing method of this polypeptide which grows the transgenic plant which holds a recombinant DNA given in (7), is made to generate and accumulate the polypeptide of a publication in any 1 term of (1) - (3) into this vegetation, and is characterized by extracting this polypeptide from the inside of this vegetation.

(20) (4) Manufacturing method of this polypeptide characterized by compounding the polypeptide in which this DNA carries out a code by imprint / translation system in vitro using DNA given in any 1 term of - (6).

[0027] (21) (1) Antibody which recognizes the polypeptide of a publication in any 1 term of - (3).

(22) (4) The oligonucleotide or this nucleotide which has the array which consists of 5 by which any 1 term of - (6) was followed in the base sequence of DNA of a publication - 60 base, and oligonucleotide which has a complementary array.

(23) How to detect the manifestation including carrying out hybridization to any 1 term of - (6), using an oligonucleotide DNA of a publication, or given in (4) (22) as a probe of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).

[0028] (24) How to detect the manifestation including performing polymerase chain reaction using the oligonucleotide given in (22) as a primer of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3).

(25) How to detect the variation of DNA which carries out the code of the polypeptide given in any 1 term of (1) - (3) by the hybridization method using an oligonucleotide DNA of a publication, or given in (4) (22) in any 1 term of - (6).

[0029] (26) How to detect the variation of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) which includes performing polymerase chain reaction using an oligonucleotide given in (22).

(27) infection -- inflammation -- following -- a disease -- being unusual -- a smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual -- fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an organization -- following -- a disease -- the pancreas -- a beta cell -- a failure -- following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual -- cell proliferation -- following -- a disease -- detecting -- a sake -- using -- ( -- 23 -- ) -- ( -- 26 -- ) -- some -- one -- a term -- a publication -- an approach .

[0030] (28) The active chronic hepatitis with which the disease accompanied by infection or inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B, Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and the disease accompanied by activation of unusual synovial membrane tissue is rheumatic arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is osteoporosis. The approach given in (27) the disease accompanied by activation of unusual immunocyte is allergy, atopy, asthma, pollinosis, respiratory tract irritation, or an autoimmune disease, and the disease accompanied by unusual cell proliferation is acute myelogenous leukemia or a malignant tumor.

[0031] (29) How to control the imprint of DNA which carries out the code of the polypeptide of a publication to any 1 term of (1) - (3) characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6), or the translation of mRNA.

(30) How to acquire the promoterregion and the imprint regulatory region of DNA which are characterized by using an oligonucleotide DNA of a publication, or given in (4) (22) for any 1 term of - (6) and which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3).

[0032] (31) (1) Physic which contains the polypeptide of a publication in any 1 term of - (3).

(32) (4) Physic which contains the recombinant vector of a publication in any 1 term of DNA given in any 1 term of - (6), (8), or (9).

(33) Physic containing an antibody given in (21).

(34) Physic containing an oligonucleotide given in (22).

[0033] (35) Physic given in (31) characterized by a polypeptide having an immunity activation operation.

(36) Physic given in (35) characterized by guiding antitumor activity and antiviral activity through an immunity activation operation.

(37) The disease accompanied by infection or inflammation in physic, the disease accompanied by differentiation growth of an unusual smooth muscle cell, The disease accompanied by activation of unusual fibroblast, the disease accompanied by activation of unusual synovial membrane tissue, The disease accompanied by the failure of a pancreas beta cell, the disease accompanied by activation of an unusual osteoclast, being unusual -- immunocyte -- activation -- following -- a disease -- being unusual -- cell proliferation -- following -- a disease -- or -- a nerve cell -- a failure -- being based -- a disease -- a therapy -- and/or -- prevention -- a

sake -- physic -- it is -- (- 32 --) - (- 34 --) -- some -- one -- a term -- a publication --  
 physic .  
 [0034] (38) physic -- infection -- inflammation -- following -- a disease -- being unusual -- a  
 smooth muscle cell -- differentiation -- growth -- following -- a disease -- being unusual --  
 fibroblast -- activation -- following -- a disease -- being unusual -- a synovial membrane -- an  
 organization -- activation -- following -- a disease -- the pancreas -- a cell -- a failure --  
 following -- a disease -- being unusual -- an osteoclast -- activation -- following -- a disease --  
 -- being unusual -- immunocyte -- activation -- following -- a disease -- or -- being unusual --  
 -- cell proliferation -- following -- a disease -- a diagnosis -- a sake -- physic -- it is -- (- 32  
 --) - (- 34 --) -- some -- one -- a term -- a publication -- physic .

[0035] (39) The active chronic hepatitis with which the disease accompanied by infection or  
 inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B,  
 Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive  
 heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent  
 diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease  
 accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the  
 restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and  
 the disease accompanied by activation of unusual synovial membrane tissue is rheumatic  
 arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell  
 is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is  
 osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy,  
 asthma, Physic of (37) or (38) publications whose disease accompanied by unusual cell  
 proliferation are pollinosis, respiratory tract irritation, or an autoimmune disease, and is acute  
 myelogenous leukemia or a malignant tumor and whose disease based on the failure of a nerve  
 cell is an Alzheimer disease or ischemic encephalopathy.

[0036] (40) (1) It is characterized by using the polypeptide of a publication for any 1 term of -  
 (3). The disease accompanied by infection or inflammation, the disease accompanied by  
 differentiation growth of an unusual smooth muscle cell, the disease accompanied by activation  
 of unusual fibroblast, The disease accompanied by activation of unusual synovial membrane  
 tissue, the disease accompanied by a pancreas beta cell failure, the disease accompanied by  
 activation of an unusual osteoclast, The medicinal screening approach for the therapy of the  
 disease accompanied by activation of unusual immunocyte, the disease accompanied by unusual  
 cell proliferation, or the disease based on the failure of a nerve cell, and/or prevention.

[0037] (41) The active chronic hepatitis with which the disease accompanied by infection or  
 inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B,  
 Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive  
 heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent  
 diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease  
 accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the  
 restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and  
 the disease accompanied by activation of unusual synovial membrane tissue is rheumatic  
 arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell  
 is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is  
 osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy,  
 asthma, The medicinal screening procedure given [ are pollinosis, respiratory tract irritation, or  
 an autoimmune disease, and the given disease accompanied by unusual cell proliferation is acute  
 myelogenous leukemia or a malignant tumor ] in (40) the given disease based on the failure of a  
 nerve cell is an Alzheimer disease or ischemic encephalopathy.

[0038] (42) Physic which acts on a polypeptide given in any 1 term of (1) - (3) obtained by the  
 screening approach (40) or given in (41) specifically.  
 (43) It is characterized by using the promoterregion and the imprint regulatory region of DNA  
 which carry out the code of the polypeptide of a publication to any 1 term of (1) - (3) obtained  
 -by the approach given in (30). The disease accompanied by infection or inflammation, the disease  
 accompanied by differentiation growth of an unusual smooth muscle cell, the disease

accompanied by activation of unusual fibroblast, The disease accompanied by activation of  
 unusual synovial membrane tissue, the disease accompanied by a pancreas beta cell failure, the  
 disease accompanied by activation of an unusual osteoclast, The medicinal screening approach  
 for the therapy of the disease accompanied by activation of unusual immunocyte, the disease  
 accompanied by unusual cell proliferation, or the disease based on the failure of a nerve cell,  
 and/or prevention.

[0039] (44) The active chronic hepatitis with which the disease accompanied by infection or  
 inflammation is represented by microorganism infection, HIV infection, and chronic hepatitis B,  
 Rheumatoid arthritis, glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive  
 heart failure, The endotoxin shock, septicemia, graft versus host disease, insulin dependent  
 diabetes mellitus, It is traumatic brain injury or inflammatory bowel disease, and the disease  
 accompanied by differentiation growth of an unusual smooth muscle cell is arteriosclerosis or the  
 restenosis. The disease accompanied by activation of unusual fibroblast is the fibroid lung, and  
 the disease accompanied by activation of unusual synovial membrane tissue is rheumatic  
 arthritis or hypertrophic arthritis. The disease accompanied by the failure of a pancreas beta cell  
 is diabetes mellitus, and the disease accompanied by activation of an unusual osteoclast is  
 osteoporosis. The disease accompanied by activation of unusual immunocyte Allergy, atopy,  
 asthma, The medicinal screening approach given in (43) that it is pollinosis, respiratory tract  
 irritation, or an autoimmune disease, the disease accompanied by unusual cell proliferation is  
 acute myelogenous leukemia or a malignant tumor, and the disease based on the failure of a  
 nerve cell is an Alzheimer disease or ischemic encephalopathy.

[0040] (45) Physic which acts on the promoterregion and the imprint regulatory region of DNA  
 which are obtained by the screening approach (43) or given in (44), and which carry out the code  
 of the polypeptide of a publication to any 1 term of (1) - (3) specifically.

(46) The immunological detecting method of a polypeptide given in any 1 term of (1) - (3)  
 characterized by using an antibody given in (21).

(47) The immunity staining method characterized by detecting the polypeptide of a publication in  
 any 1 term of (1) - (3) using an antibody given in (21).

[0041] (48) How to screen the matter which controls or promotes the imprint or translation of  
 DNA which is characterized by using an antibody given in (21), and which carries out the code of  
 the polypeptide of a publication to any 1 term of (1) - (3).

(49) (1) The manifestation of DNA which carries out the code of the polypeptide of a publication  
 to any 1 term of - (3) is a part or the knock out nonhuman animal controlled completely.

(50) (1) The activity which the polypeptide of a publication has in any 1 term of - (3) is a part or  
 the knock out nonhuman animal controlled completely.

[0042] (51) The screening approach of a variant polypeptide of having dominant negative activity  
 to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1)(1) characterized  
 by using polypeptide of publication for any 1 term of - (3) - (3).

(52) the variant polypeptide which has dominant negative activity to NF-kappa B activation of  
 the polypeptide of a publication in any 1 term of acquisition \*\*\*\* and (1) - (3) by the screening  
 approach given in (51).

(53) DNA which carries out the code of the variant polypeptide given in (52).

[0043] (54) The screening approach of a variant polypeptide of having the variation which is  
 characterized by using the polypeptide of a publication for any 1 term of - (3) and which raises  
 this activation to NF-kappa B activation of the polypeptide of a publication in any 1 term of (1)  
 (1) - (3).

(55) The variant polypeptide which is acquired by the screening approach given in (54) and to  
 which the NF-kappa B activation ability of the polypeptide of a publication went up in any 1 term  
 of (1) - (3).

(56) DNA which carries out the code of the variant polypeptide given in (55).

[0044]

[Embodiment of the Invention] In the amino acid sequence chosen from the group which consists  
 of an amino acid sequence expressed with the polypeptide 2. array numbers 1-5 which have the  
 amino acid sequence chosen from the group which consists of an amino acid sequence

expressed with either of 1. array numbers 1-5 as a polypeptide of this invention one or more amino acid deletion. The amino acid sequence chosen from the group which consists of an amino acid sequence expressed with either of the polypeptide 3. array numbers 1-5 which has the activity which it consists [activity] of an amino acid sequence permuted and/or added, and raises the activity of NF-kappa B, and the amino acid sequence which has 60% or more of homology are included. And the polypeptide which has the activity which raises the activity of NF-kappa B can be mentioned.

[0045] The polypeptide which has the amino acid sequence to which one or more amino acid was \*\*\*\*(ed), permuted and/or added in the polypeptide which has the above-mentioned amino acid sequence Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, 1989 (it abbreviates to the 2nd edition of molecular cloning hereafter). Current Protocols in Molecular Biology, John Wiley & Sons, 1987-1997 (it abbreviates to current PUROTO call Inn molecular biology hereafter) Nucleic Acids Research, 10, and 6487 (1982), Proc.Natl.Acad.Sci., USA, 79, and 6409 (1982), Gene, 34, 315 (1985), Nucleic Acids Research, 13, 4431 (1985). The site-specific mutation introducing method of a publication is used for Proc.Natl.Acad.Sci. USA, 82, 488 (1985), etc. For example, it can carry out by introducing site-specific mutation into DNA which carries out the code of the polypeptide which has one amino acid sequence of the array numbers 1-5, although the number of deletion and the amino acid permuted and/or added comes out of 1 partly, and there is and especially the number is not limited --- the technique of common knowledge, such as the above-mentioned site-specific mutation introducing method, --- the number of deletion and extent which can be permuted or added --- it is --- for example, 1- dozens of pieces are 1-5 pieces still more preferably 1-10 pieces more preferably 1-20 pieces.

[0046] Moreover, as a polypeptide of this invention, the amino acid sequence of a publication and the amino acid sequence which has 60% or more of homology are included in either of the array numbers 1-5. The homology with an amino acid sequence given in either of the array numbers 1-5 With analysis software, such as BLAST [J.Mol.Biol., 215, and 403 (1990)] and FASTA (Methods in Enzymology, 183, 63-69) It is most preferably [97% or more of] more preferably desirable [70% or more / 80% or more] at least 60% or more, when it calculates using a default (initialization) parameter 95% or more especially preferably 90% or more still more preferably preferably.

[0047] DNA which has the base sequence expressed with either of the DNA3. array numbers 6-10 which are DNA of the DNA2. claim 4 publication which carries out the code of the polypeptide of a publication to any 1 term of 1. claims 1-3 as DNA of this invention, and DNA hybridized under stringent conditions, and carry out the code of the polypeptide which has the activity which raises the activity of transcription factor NF-kappa B can be mentioned.

[0048] Since two or more sorts of gene codes generally exist per amino acid, it is contained in DNA of this invention, if the code of the polypeptide of this invention is carried out even if either of the array numbers 6-10 is DNA which has a different base sequence. With DNA hybridized under stringent conditions For example, DNA of this inventions, such as DNA which has the base sequence expressed with the array numbers 6, 7, 8, 9, or 10, or some of its fragments are used as a probe. DNA obtained by using a colony hybridization method, a plaque hybridization method, or a Southern blotting hybridization method is meant. The filter which fixed DNA of a colony or the plaque origin is specifically used. The SSC solution of 0.1 - 2 double concentration the bottom of the sodium chloride existence of 0.7 - 1.0 mol/l, and after performing hybridization at 85 degrees C (the SSC solution of concentration 1 time) DNA which can be identified by washing a filter under 65-degree-C conditions can be mentioned using a 150 mmol/l sodium chloride and 15 mmol/l sodium-citrate twist. Hybridization is the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, and D NACloning 1. : It can carry out according to the approach indicated by Core Techniques, A Practical Approach, Second Edition, Oxford University, and 1995 grades.

[0049] As DNA which can be hybridized, specifically When it calculates with analysis software, such as BLAST and FASTA, using a default (initialization) parameter The base sequence expressed with the array numbers 6, 7, 8, 9, or 10, and DNA which has at least 60% or more of

homology. DNA which has 98% or more of homology most preferably can be mentioned especially 95% or more preferably 90% or more still more preferably 80% or more 70% or more.

[0050] Hereafter, this invention is explained to a detail.

1. Preparation Homo sapiens mRNA of DNA of this invention may use a commercial thing (for example, product made from Clontech), and may prepare from human tissue as the following, as the approach of preparing all RNA from an organization --- thiocyanic acid guanidine - trifluoroacetic acid caesium method [Methods in Enzymology, 154, and 3] (19 87) acidity thiocyanic acid guanidine phenol chloroform (AGPC) --- law [Analytical Biochemistry, 162, 156 (1987), the experimental medicine, 9, and 1937 (1991)] etc. is mentioned. Moreover, as an approach of preparing mRNA as polyA+RNA from all RNA, the oligo (dT) fixed cellulose column method (the 2nd edition of molecular cloning) etc. is mentioned. Furthermore, FastTrack mRNA Isolation Kit (product made from Invitrogen), Quick Prep mRNA mRNA can be prepared by using kits, such as Purification Kit (product made from Pharmacia).

[0051] A cDNA library is produced from prepared human tissue mRNA. As a cDNA library producing method, the 2nd edition of molecular cloning, Current PUROTO call Inn molecular biology, A Laboratory Manual, 2nd Ed., the approach indicated by 1989 grades, (Or a commercial kit, for example, SuperScript Plasmid System for cDNA, Synthesis and Plasmid Cloning (product made from Life Technologies)) The approach using ZAP-cDNA Synthesis Kit (product made from STRATAGENE) etc. is mentioned.

[0052] As a cloning vector for producing a cDNA library, if independence reproduction can be carried out in Escherichia coli K-12, a phage vector, a plasmid vector, etc. can use either. Specifically The product made from ZAP Express[STRATAGENE, Strategies, 5, 58 (1992)], and pBluescript II SK --- (+ [Nucleic Acids Research, 17, and 9494 (1989)]) --- Lambda ZAP II (product made from STRATAGENE), lambda dgt10, and lambda dgt11 [DNA cloning, A Practical Approach, 1, and 49 (1985)], lambda Triplex (product made from Clontech), lambda ExCell (product made from Pharmacia), pTT7318U (product made from Pharmacia), pcD2[Mol.Cell.Biol., 3, 280 (1983)], pUC18 [Gene, 33, and 103 (1985)], etc. can be mentioned.

[0053] Either can be used if it is a microorganism belonging to Escherichia coli as a host microorganism. Specifically The product made from Escherichia coli XL1-Blue MRF[STRATAGENE, Strategies, 5, 81 (1992)], and Escherichia coli C600 [Genetics, 39, and 440 (1954)], Escherichia coli Y1088 [Science, 222, and 778 (1983)], Escherichiacoli Y1090 [Science, 222, and 778 (1983)], Escherichia coli NM522 [J.Mol.Biol., 166, and 1 (1983)], Escherichia coli K802 [J.Mol.Biol., 16, and 118 (1966)], Escherichia coli JM105 [Gene, 38, and 275 (1985)], etc. are used.

[0054] Although this cDNA library may be used for the following analyses as it is, in order to lower the rate of the imperfect length cDNA and to acquire the perfect length cDNA efficiently if possible Oligo-capping method [Gene which Sugano and others developed, 138, and 171 (1994), Gene, 200, 149 (1997), a protein nucleic-acid enzyme, 41, and 603 (1996), The experimental medicine, 11, 2491 (1993), and cDNA cloning, Yodosha (1996) Method of producing a gene library, Yodosha (1994) The cDNA library prepared using ] may be used for the following analyses. [0055] The base sequence of this DNA is determined by isolating each clone from the produced cDNA library, and analyzing the base sequence of cDNA from an end using base sequence analysis apparatus, such as the base sequence analysis approach usually used, for example, the dideoxy chain termination method of Sanger and others (Sanger), [Proc.Natl.Acad.Sci.USA, 74, 54 63 (1977)], and ABI PRISM377 DNA sequencer (product made from PE Biosystem), about each clone. By translating the acquired base sequence into an amino acid sequence, the amino acid sequence of the polypeptide in which this DNA carries out a code can be acquired.

[0056] Moreover, the base sequence from which the acquired base sequence was acquired [whether it is a new base sequence and ], and a base sequence with homology can be searched in by comparing the acquired base sequence using homology analyzers, such as a base sequence in base sequence databases, such as GenBank and EMBL, BLAST, and FASTA. Moreover, the family protein suddenly presumed also in the polypeptide in which the base sequence carries out a code and a polypeptide with homology, for example, the polypeptide originating in the corresponding gene in living thing kind with another rat, the same activity, and the same



function can be searched by comparing the amino acid sequence acquired from the base sequence with amino acid sequence databases, such as SwissProt, PIR, and GenPept. [0057] Based on the base sequence of the homologous gene which became clear by database retrieval, a specific primer is designed in this gene and PCR is performed by using as mold the single strand cDNA acquired as mentioned above or a cDNA library. When a magnification fragment is obtained, subcloning of this fragment is carried out to a suitable plasmid, subcloning -- a magnification fragment -- as it is -- or a restriction enzyme and DNA polymerase -- after processing and a law -- it can carry out by including in a vector by the method. As a vector, pBluescript SK (-), (the product made from Stratagene), pBluescript II SK (+), (the product made from Stratagene), pDIRECT [Nucleic Acid's Research, 18, and 6069 (1990)], pCR-Script Amp SK (+), (the product made from Stratagene), p7Blue (product made from Novagen), pCRII (product made from Invitrogen), pCR-TRAP (product made from Genehunter), pNo TAT7 (5'→3' company make), etc. can be mentioned.

[0058] After DNA which consists of one base sequence of the array numbers 6-10 is once acquired and the base sequence is determined, DNA of this invention is acquirable by preparing the primer based on the base sequence of 5' edge and 3' edge of this base sequence, and amplifying DNA using cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal.

[0059] Moreover, DNA of this invention is acquirable by performing colony hybridization and plaque hybridization (the 2nd edition of molecular cloning) to cDNA or the cDNA library compounded from mRNA contained in the tissue or the cell of Homo sapiens or a nonhuman animal by using as a probe an overall length or a part of DNA which consists of one base sequence of the array numbers 6-10.

[0060] DNA of this invention is also acquirable by carrying out chemosynthesis based on the base sequence of determined DNA with DNA synthesis machines, such as a DNA synthesis machine (model 392) of Perkin-Elmer using a HOSUFO aminodite method. As an oligonucleotide of this invention, the derivative (henceforth, derivative oligonucleotide) of oligonucleotides, such as Oligo DNA and Oligo RNA, and this oligonucleotide etc. is mentioned.

[0061] As this oligonucleotide or this oligonucleotide, and the oligonucleotide (henceforth, antisense oligonucleotide) equivalent to a complementary array -- for example, in some base sequences of mRNA to detect, the sense primer equivalent to the base sequence by the side of a five prime end, the antisense primer equivalent to the base sequence by the side of a three--dash terminal, etc. can be mentioned. However, the base which is equivalent to a uracil in mRNA serves as thymidine in an oligonucleotide primer.

[0062] As a sense primer and an antisense primer, it is the oligonucleotide which does not change extremely both melting out temperature (Tm) and number of bases, and the thing of the number of 10 - 50 bases is mentioned preferably five to 60 base. What was exchanged for HOSUFO thioate association in the phosphodiester bond in an oligonucleotide as a derivative oligonucleotide. That from which the phosphodiester bond in an oligonucleotide was changed into N3'-P5' HOSUFO friend date association, That from which RIPOSU and the phosphodiester bond in an oligonucleotide were changed into peptide nucleic-acid association, That by which the uracil in an oligonucleotide was permuted by the C-5 propynyl uracil, That by which the cytosine in an oligonucleotide was permuted by the C-5 thiazole uracil, That by which the cytosine in an oligonucleotide was permuted with the C-5 propynyl cytosine, That by which the cytosine in an oligonucleotide was permuted with the phenoxazine qualification cytosine (phenoxazine-modified cytosine). That by which the ribose in an oligonucleotide was permuted by the 2'-methoxyethoxy ribose is mentioned [a cell technology, 16, and 1463 (1997)].

[0063] 2. In host cell this invention used for the detecting method (1) activity detection of NF-kappa B activation of DNA of this invention, if it is the cell which can introduce DNA into intracellular as a host cell used in order to detect the activity of DNA, any cells can be used. As this cell, the cell originating in for example, bacteria and Archea, algae, a fungus, vegetation, an animal, etc. is mentioned. Specifically, the cell of the following living thing origin is mentioned. [0064] Escherichia coli, Bacillus subtilis, etc. are mentioned as bacteria and Archea. The cyanobacterium of a Synechococcus group or a Synechocystis group etc. is mentioned as algae.

As vegetation, tobacco, Arabidopsis, a tomato, a potato, the rapeseed, cotton, soybeans, a rice, or corn is mentioned. Saccharomyces cerevisiae, Aspergillus niger, etc. are mentioned as a fungus. Mammalian, Arthropoda, etc. are mentioned as an animal.

[0065] As mammalian, Homo sapiens, an ape, a mouse, a rat, a guinea pig, or a mink is mentioned. Specifically as a human cell, the T cell stock Jurkat (the cell strain of number TIB-512 of an American type culture collection (it is hereafter written as ATCC)), the B cell stock Namalwa (ATCC CRL-1432), the uterine cancer cell strain Hela (ATCC CCL-2), the nephrocyte stock 293 [J.Gen.Vol.36 and 59-72 (1977)], etc. can be used. As a cell of mammals other than Homo sapiens, ape nephrocyte stock COS-1 (ATCC CRL-16 50), Ape nephrocyte stock COS-7 (ATCC CRL-1651), the Chinese hamster ovary cell (Chinese Hamster Ovary) cell strain CHO (ATCC CRL-9096, ATCC CCL-61), Mouse cell strain Ba/F3 (RIKEN Cell Bank RCB0805), The mouse cell strain L929 (RIKEN Cell Bank RCB0081), rat cell strain NRK-49F (ATCC CRL-1570), the mink cell strain MvLu (ATCC CCL-64), etc. can be used. A silkworm is mentioned as Arthropoda. Specifically, nine shares of Spodoptera frugiperda Sf, 21 shares of Sf(s), etc. can be used. When retrieval of DNA used as the screening target of the protein nature drugs for a therapy or drugs is the purpose, it is desirable to make the cell of mammalian, especially a human cell into a host.

[0066] (2) If it is the approach of introducing a gene into a host cell as an approach of introducing DNA of transgenics method this invention to a host cell into a host cell, it can use by any approaches. For example, the electroporation method (the Yodoshia biotechnology manual series 4 and 23), A calcium phosphate method (the Yodoshia biotechnology manual series 4 and 16), The RPOFE cushion method (the Yodoshia biotechnology manual series 4 and 28), A microinjection method (the Yodoshia biotechnology manual series 4 and 36), Well-known approaches, such as the adenovirus method (the Yodoshia biotechnology manual series 4 and 43) and the vaccinia virus method (Yodoshia biotechnology manual series 4 and 59) retrovirus vector method (the Yodoshia biotechnology manual series 4 and 74), can be used.

[0067] (3) Since DNA of approach this invention which acquires DNA of this invention can activate NF-kappa B by making it discovered in a cell, it can acquire DNA of this invention by using the approach of detecting activation of NF-kappa B in a cell. The following approaches are mentioned as an approach of detecting activation of NF-kappa B.

[0068] For example, the approach of analyzing association to imprint regulatory region by the gel shifting method (the Yodoshia biotechnology manual series 5 and 107) etc., and the method of detecting the phosphorylation of IkappaB and ubiquitination by western blotting (the Yodoshia biotechnology manual series 7 and 179) etc. are mentioned as an approach using a cell extract. Furthermore, the approach of detecting using a reporter gene as an approach of detecting efficiently can be mentioned. As a reporter gene, the gene which carries out the code of luciferase, the Homo sapiens placenta alkaline phosphatase, the beta-galactosidase, urokinase, chloramphenicol acetyltransferase, a human growth hormone, various Greenfluorescent protein (following, GFP), etc. can be used. If it is the promoter who is imprinted by NF-kappa B and gets as a promoter who connects with a reporter gene, any promoters can use. For example, the promoter DNA fragment isolated by starting the promoterregion of a gene where the manifestation is controlled by activation of NF-kappa B by restriction enzyme digestion from Chromosome DNA, the promoter DNA fragment obtained by amplifying by the PCR method by using Chromosome DNA as mold, or the synthetic DNA fragment which has this promoter's base sequence is mentioned.

[0069] Specifically IL-1alpha, IL-1beta, IL-2, IL-3, IL-6, IL-8, IL-12, TNF-alpha, TNF-beta, IFN-beta, M-CSF, GM-CSF, G-CSF, L-2Ralpha, Ig-kappa-LC, T-cell receptorbeta, the MHC class 1, beta 2-microglobulin, LAM-1, VCAM-1, ICAM-1, blood serum amyloid A precursor protein, Angiotensinogen, the complement factor B, the complement factor C3, the complement factor C4, iNOS, COX-2, VEGF-R2, c-Rel, p105, IkappaBalpha, Promotors, such as c-Myc, IRF-1, HIV-1, HIV-2, SIVmac, CMV, HSV-1, SV40, and adenovirus, a synthetic promoter with [ one or more ] those consensus sequences, etc. are mentioned.

[0070] By the detection approach using a reporter gene, after producing the imprint unit which

connected the reporter gene with the above-mentioned promoter, the cell strain which included the imprint unit in the chromosome of a host cell is produced. After introducing into intracellular [this] the unit which discovers DNA of this invention and making DNA of this invention discover, activation of NF-kappa B is detectable by measuring the amount of manifestations of a reporter gene. Or after producing the imprint unit which connected the reporter gene with the above-mentioned promoter, activation of NF-kappa B is detectable by introducing into coincidence two units, this imprint unit and the unit which discovers DNA of this invention, at a host cell, and measuring the amount of manifestations of a reporter gene.

[0071] 3. Using the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc., by the following approaches, it can be made discovered in a host cell and the polypeptide of manufacture this invention of the polypeptide of this invention can manufacture DNA of this invention.

[0072] The DNA fragment of the suitable die length containing the part which carries out the code of this polypeptide if needed based on an overall length cDNA is prepared. A recombination vector is produced by inserting this DNA fragment or an overall length cDNA in the lower stream of a river of the promoter of a suitable expression vector. The transformant which produces the polypeptide of this invention can be obtained by introducing this recombination vector into the host cell which suited this expression vector.

[0073] As a host cell, if bacteria, yeast, an animal cell, an insect cell, a plant cell, etc. can discover the gene made into the purpose, all can use them. As an expression vector, in the above-mentioned host cell, the nest to the inside of a chromosome is possible, and autonomous replication's being possible or the thing containing a promoter is used for the location which can imprint DNA which carries out the code of the polypeptide of this invention.

[0074] When using prokaryotes, such as bacteria, as a host cell, while the recombination vector which comes to contain DNA which carries out the code of the polypeptide of this invention can be replicated autonomously in a prokaryote, it is desirable that they are a promoter, a ribosome junction sequence, the gene that carries out the code of the polypeptide of this invention, and the vector which consisted of conclusion arrays of an imprint. In addition, the gene which controls a promoter may be contained in the vector.

[0075] As an expression vector, for example pBTrp2 (product made from Boehringer Mannheim), pBTac1 (product made from Boehringer Mannheim), pBTac2 (product made from Boehringer Mannheim), pKK 233-2 (product made from Pharmacia), pSE280 (product made from Invitrogen), pGEMEX-1 (product made from Promega), pOE-8 (product made from QIAGEN), pKYP10 (Provisional-Publication-No. 5 8-110600 No.) and pKYP200 [Agricultural Biological Chemistry, 48, and 669 (1984)], pLSA1 [Agric.Bil o.Chem., 53, and 277 (1989)], pGEL1 [Proc.Natl.Acad.Sci.USA, 82, and 4306 (1985)], pBluescript II SK (-), (the product made from Stratagene), From pTrs30[Escherichia coli JM109/pTrs30 (FERM BP-5407), preparation], From pTrs32[Escherichia coli JM109/pTrs32 (FERM BP-5408), preparation], It prepares from pGHA2 [Escherichia coli IGHA2 (FER M BP-400). It prepares from JP.60-221091,A] and pGKA2 [Escherichia coli IGKA2 (FERM BP-6798). JP.60-221091,A] and pTerm2 (U.S. Pat. No. 4,686,191 --) U.S. Pat. No. 4,939,094 and U.S. Pat. No. 5,160,735, pSupex, and pUB110, pTP5, pC194 and pEG400 [JBacteriol., 172, and 2392 (1990)]. As a . . . expression vector which can mention pGEX (product made from Pharmacia), a pET system (product made from Novagen), etc. It is desirable to use what adjusted between the Shine-Dalgarno (Shine-Dalgarno) arrays and initiation codons which are a ribosome junction sequence in a suitable distance (for example, six to 18 base).

[0076] As a promoter, as long as it can be discovered in a host cell, what kind of thing may be used. For example, the promoter originating in Escherichia coli, phage, etc., such as a trp promoter (P<sub>trp</sub>), a lac promoter, P<sub>L</sub> promoter, P<sub>R</sub> promoter, and T7 promoter, and SPO1 promoter, SPO2 promoter, a penP promoter, etc. can be mentioned. Moreover, the promoter by whom the design alteration was artificially done like the promoter (P<sub>trp</sub>x2) who did 2 serials of the P<sub>trp</sub>, a tac promoter, lacT7 promoter, and a let promoter [Gene, 44, and 29 (1986)] can use.

[0077] The production rate of the polypeptide made into the purpose can be raised by permuting a base so that it may become the optimal codon for a host's manifestation about the base sequence of the part which carries out the code of the polypeptide of this invention. In the

recombination vector of this invention, although the conclusion array of an imprint is not necessarily required for the manifestation of DNA of this invention, it is desirable to arrange the conclusion array of an imprint directly under a structural gene.

[0078] As a host cell, Escherichia, Serratia, Bacillus, Brevibacterium, The microorganism belonging to Corynebacterium, Microbacterium, Pseudomonas, etc., For example, Escheri chia coli XL1-Blue, Escherichia coli XL2-Blue, Escherichia coli DH1, Escherichia coli MC1000, Escherichia coli KY3276, Escherichia coli W1485, and Escherichia coli JM109, Escherichia coli HB101, Escherichia coli No.49, Escherichia coli W3110 and Escherichia coli INY49, Serr atia ficaria, Serratia fonticola, Serratia liquefaciens, Serratia marcescens, Bacillus subtilis, Bacillus amyloliquefaciens, Brevibacterium ammoniagenes, Brevibacterium immariophilum ATCC14088 and Brevibacterium saccharolyticum ATCC14066, Brevibacterium flavum ATCC14067.

Brevibacterium lactofermentum ATCC13869, and Corynebacterium glutamicum ATCC1303 2, Microbacterium ammoniophilum ATCC15354, and Pseudomonas sp.D-0110 grade can be mentioned.

[0079] All can be used if it is the approach of introducing DNA to the above-mentioned host cell as the introductory approach of a recombination vector. For example, the approach using calcium ion [Proc.Natl. Acad.Sci.USA, 69, and 2110 (1972)]. The approach of a publication etc. can be mentioned to the protoplast method [JP.63-248394,A] or Gene, 17, 107 (1982) and Mole cular & General Genetics, 168, and 111 (1979).

[0080] When using yeast as a host cell, YEP13 (ATCC37115), YEP24 (ATCC37051), YOp50 (ATCC37419), pHS19, and pHS15 grade can be mentioned as an expression vector. As a promoter, as long as it can be discovered in a yeast-fungus stock, which thing may be used, for example, they are the promoter of the gene of glycolytic pathways, such as a hexose kinase, PHO5 promoter, a POK promoter, a GAP promoter, an ADH promoter, gal1 promoter, gal10 promoter, a heat shock protein promoter, and MF1. A promoter, CUP1 promoter, etc. can be mentioned.

[0081] As a host cell, the microorganism belonging to a Saccharomyces, a clew IBERO married-woman group, the Trichosporon, a SHUWANIO married-woman group, etc., for example,

Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis, Trichosporon pullulans, Schwannomyces alluvius, etc. can be mentioned. All can be used if it is the approach of introducing DNA into yeast as the introductory approach of a recombination vector. For example, the electroporation method [Methods.Enzymol., 194, and 182 (1990)]. The spheroplast method [Proc.Natl.Acad.Sci.USA, 84, and 1929 (1978)]. The acetic-acid lithium method [JBacteriology, 153, and 163 (1983)], an approach given in [Proc.Natl.Acad.Sci.USA, 75, and 1929 (1978)], etc. can be mentioned.

[0082] In using an animal cell as a host, as an expression vector For example, pcDNA1, pcDM8 (Funakoshi Co., Ltd. make), pAGE107 [JP.3-22979,A,Cytotechnology, 3, and 1 33 (1990)], pAS 3-3 (JP.2-227075,A) and pCDM8 [Nature, 329, and 840 (1987)], pcDNA1/A mp (product made from Invitrogen), pREP4 (product made from Invitrogen) and pAGE103 [JBiochemistry, 101, and 1307 (1987)], and pAGE210 grade can be mentioned.

[0083] As a promoter, if it can be discovered in an animal cell, all can be used, for example, the promoter of IE (immediate early) gene of a cytomegalovirus (CMV), the initial promoter of SV40, the promoter of a retrovirus, a metallothionein promoter, a heat shock promoter, SRalpha promoter, etc. can be mentioned. Moreover, the enhancer of Homo sapiens's CMV IE gene may be used with a promoter.

[0084] As a host cell, the NAMARUBA (Nimalwa) cell which is a human cell, the COS cell which is a cell of an ape, the CHO cell which is a cell of a Chinese hamster, HBT5637 (JP.63-299,A), etc. can be mentioned. If it is the approach of introducing DNA into an animal cell as the introductory approach of a recombination vector, all can be used, for example, the electroporation method [Cytotechnology, 3, and 133 (1990)], a calcium phosphate method (JP.2-227075,A), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, and 7413 (1987)], etc. can be mentioned.

[0085] When using an insect cell as a host, the polypeptide of this invention can be discovered by the approach indicated by the current PUROTO call Inn molecular biology supplement 1-38 (1



987-1997). Baculovirus Expression Vectors, A Laboratory Manual. W.H.Freeman and Company, New York (1992). Bio/Technology, 6, 47, etc. (1988).

[0086] That is, after carrying out cotransduction of a recombinant gene installation vector and the baculovirus to an insect cell, rearranging in insect cell culture supernatant liquid and obtaining a virus, it can rearrange further, a virus can be infected with an insect cell, and the polypeptide of this invention can be made to discover. As a transgenics vector used in this approach, pVL1392, pVL1393, pBlueBacIII (both product made from Invitrogen), etc. can be mentioned, for example.

[0087] As a baculovirus, the out GURAFKA KARIFORUNIKA NUKUREA poly sludge cis- virus (Autographa californica nuclear polyhedrosis virus) which is a virus infected with the department insect of a cutworm can be used, for example. As an insect cell, Sf9 and Sf21 which are the ovarian cell of Spodoptera frugiperda [Baculovirus Expression Vectors, A Laboratory Manual, W.H.Freeman and Company, and New York] (1992). High5 (product made from Invitrogen) which is the ovarian cell of Trichoplusia ni can be used.

[0088] As the cotransduction approach of the above-mentioned recombinant gene installation vector to an insect cell and the above-mentioned baculovirus for preparing a recombinant virus, a calcium phosphate method (JP.2-2270.A 75), the RIPOFE cushion method [Proc.Natl.Acad.Sci.USA, 84, 7413 (1987)], etc. can be mentioned, for example. When using a plant cell as a host cell, a Ti plasmid, a tobacco mosaic virus vector, etc. can be mentioned as an expression vector.

[0089] As a promoter, if it can be discovered in a plant cell, which thing may be used, for example, 35S promoter of a cauliflower mosaic virus (CaMV), rice actin 1 promoter, etc. can be mentioned. As a host cell, plant cells, such as tobacco, a potato, a tomato, a ginseng, soybeans, rape, alfalfa, a rice, wheat, and a barley, etc. can be mentioned.

[0090] If it is the approach of introducing DNA into a plant cell as the introductory approach of a recombinant vector, all can be used, for example, Agrobacterium (Agrobacterium) (JP.59-140885.A, JP.60-70080.A, WO 94/00977), the electroporation method (JP.60-251887.A), the approach (the 2606856th patent 2517813rd of a patent) using party Kurgan (gene gun), etc. can be mentioned.

[0091] As the gene expression approach, secretory production, a fusion polypeptide manifestation, etc. can be performed according to the approach indicated by the 2nd edition of molecular cloning in addition to a direct manifestation. When it is made discovered by yeast, the animal cell, the insect cell, or the plant cell, the polypeptide to which sugar or a sugar chain was added can be obtained.

[0092] This polypeptide can be manufactured by cultivating the transformant incorporating DNA of this invention which rearranges and holds an expression vector to a culture medium, carrying out generation are recording of the polypeptide of this invention into a culture, and extracting this polypeptide from this culture. As a culture medium which cultivates the transformant obtained considering eukaryotes, such as prokaryotes, such as Escherichia coli, or yeast, as a host, the carbon source in which this living thing can carry out utilization, a nitrogen source, mineral, etc. are contained, and as long as it is the culture medium which can cultivate a transformant efficiently, any of a natural medium and a synthetic medium may be used.

[0093] Alcohols, such as organic acids, such as carbohydrates, such as a glucose, fructose, a sucrose, molasses containing these, starch, or starch hydrolysate, an acetic acid, and a propionic acid, ethanol, and propanol, etc. can be used that what is necessary is just that in which this living thing can carry out utilization as a carbon source. As a nitrogen source, the ammonium salt of inorganic acids, such as ammonia, an ammonium chloride, an ammonium sulfate, ammonium acetate, and ammonium phosphate, or an organic acid, other nitrogen-containing compounds and a peptone, a meat extract, a yeast extract, corn steep liquor, casein hydrolysate, soybean cake and soybean cake hydrolysate, various fermentation fungus bodies, the digest of those, etc. can be used.

[0094] As mineral salt, the first potassium of a phosphoric acid, the second potassium of a phosphoric acid, magnesium phosphate, magnesium sulfate, a sodium chloride, a ferrous sulfate, a manganese sulfate, a copper sulfate, a calcium carbonate, etc. can be used. Culture is usually

performed under aerobic conditions, such as shaking culture or deep part aeration spinner culture. Culture temperature has good 15-40 degrees C, and culture time amount is usually for 16 hours - seven days. pH under culture is held to 3.0-9.0. Adjustment of pH is performed using an inorganic or organic acid, an alkali solution, a urea, a calcium carbonate, ammonia, etc.

[0095] Moreover, antibiotics, such as ampicillin and a tetracycline, may be added to a culture medium if needed during culture. When cultivating as a promotor the microorganism using an inducer may be added to a culture medium if needed. For example, when cultivating the microorganism which used the trp promotor for isopropyl-beta-D-thio galactopyranoside (IPTG) etc. when cultivating the microorganism using a lac promotor which was rearranged and carried out the transformation by the vector and which was rearranged and carried out the transformation by the vector, the Indore acrylic acid (IAA) etc. may be added to a culture medium.

[0096] As a culture medium which cultivates the transformant obtained considering the animal cell as a host RPMI1640 culture medium currently generally used [The Journal of the American Medical Association, 199, and 519 (1967)]. The MEM culture medium of Eagle [Science, 122, and 501 (1952)]. A Dulbecco alteration MEM culture medium [Virology, 8, and 396 (1959)]. The culture medium which added fetal calf serum etc. can be used for 199 culture media [Proceeding of the Society for the Biological Medicine, 73, and 1 (1950)] or these culture media. Culture --- usually --- pH 6-8, 30-40 degrees C, and 5%CO --- it carries out for one - seven days under lower conditions 2 \*\*\*\*. Moreover, antibiotics, such as a kanamycin and penicillin, may be added to a culture medium if needed during culture.

[0097] As a culture medium which cultivates the transformant obtained considering the insect cell as a host, the TNM-FH culture medium (product made from Pharmingen) currently generally used, a Sf-900 II SFM culture medium (product made from Life Technologies), ExCell400 and ExCell405 (all are the products made from JRH Biosciences), Grace's Insect Medium [Nature, 195, and 788 (1962)], etc. can be used. Culture is usually performed for one - five days under conditions, such as pH 6-7 and 25-30 etc. degrees C. Moreover, antibiotics, such as gentamycin, may be added to a culture medium if needed during culture.

[0098] A plant cell can be made to be able to specialize in the cell and organ of the vegetation as a cell, and the transformant obtained as a host can cultivate it. As a culture medium which cultivates this transformant, auxin, cytokinin, etc. can use the culture medium which added plant hormone for Murashige - currently generally used and - SUKUGU (MS) culture medium, the White (White) culture medium, or these culture media. Culture is usually performed for three - 60 days under pH 5-9 and 20-40-degree C conditions. Moreover, antibiotics, such as a kanamycin and hygromycin, may be added to a culture medium if needed during culture.

[0099] This approach can be chosen by there being an approach which it makes host intracellular produce, an approach of making it secrete out of a host cell, or the approach of making it produce on a host cell envelope as a process of the polypeptide of this invention, and changing the host cell to be used and the structure of a polypeptide made to produce. When the polypeptide of this invention is produced on host intracellular or a host cell envelope, Paulson's and others approach [J.Biol.Chem., 264, and 17619 (1989)], Approach [Proc.Natl.Acad.Sci.USA of a low and others, 86, and 8227 (1989)]. This polypeptide can be made to secrete positively out of a host cell by applying the approach of a publication correspondingly to Genes Develop., 4, 1288 (1990)] or JP.5-336963.A, and WO94 / 23021 grades.

[0100] That is, the polypeptide of this invention can be made to secrete positively out of a host cell by making it discovered in the form which added transit peptide before the polypeptide including the active site of the polypeptide of this invention using the transgenic technique. Moreover, according to the approach indicated by JP.2-227075.A, a volume can also be raised using the gene amplification system using a dihydrofolate reductase gene etc.

[0101] Furthermore, by making the cell of the animal which carried out transgenics, or vegetation redifferentiate, the animal individual (transgenic nonhuman animal) or vegetable individual (transgenic plant) into which the gene was introduced can be developed, and the polypeptide of this invention can also be manufactured using these individuals. When a transformant is an

animal individual or a vegetable individual, this polypeptide can be manufactured by breeding or growing, carrying out generation are recording of this polypeptide according to the usual approach, and extracting this polypeptide from this animal individual or a vegetable individual. [0102] The method of producing the polypeptide of this invention is mentioned into the animal which introduced and developed the gene as an approach of manufacturing the polypeptide of this invention using an animal individual, for example according to the well-known approach [American Journal of Clinical Nutrition, 63, 639S (1996), American Journal of Clinical Nutrition, 63, 627S (1996), Bio/Technology, 9, and 830 (1991)].

[0103] In the case of an animal individual, this polypeptide can be manufactured by breeding the transgenic nonhuman animal which introduced DNA which carries out the code of the polypeptide of this invention, generating and storing up this polypeptide into this animal, and extracting this polypeptide from the inside of this animal. As an are recording location in this animal, the milk (JP.63-309192A) of this animal, an egg, etc. can be mentioned, for example, under the present circumstances — although all can be used as a promotor boiled and used if it can be discovered for an animal — an alveolar epithelial cell — specific alpha casein promotor who is a promotor, beta casein promotor, a beta lactoglobulin promotor, a whey acidity protein promotor, etc. are used suitably.

[0104] As an approach of manufacturing the polypeptide of this invention using a vegetable individual For example, well-known approach [tissue culture and 20 (1994), the transgenic plant which introduced DNA which carries out the code of the polypeptide of this invention It grows according to tissue culture, 21 (1995), Trends in Biotechnology, 15, and 45 (1997)]. The method of producing this polypeptide is mentioned by generating and storing up this polypeptide into this vegetation, and extracting this polypeptide from the inside of this vegetation.

[0105] When the polypeptide of this invention is discovered in the state of the dissolution to intracellular, the polypeptide manufactured by the transformant of this invention collects cells according to centrifugal separation after culture termination, crushes a cell by the ultrasonic crusher, the French press, the MANTONGAURIN homogenizer, dynamill, etc. after suspending in the drainage system buffer solution, and obtains a cell-free extract. The isolation purification method of an enzyme usual from the supernatant liquid obtained by carrying out centrifugal separation of this cell-free extract, namely, the salting-out method by the solvent extraction method, an ammonium sulfate, etc., the desalting method, settling by the organic solvent, The anion-exchange chromatography method using resin, such as diethylaminoethyl (DEAE)-sepharose and DIAIONHPA-75 (Mitsubishi Kasei Corp. make). The cation-exchange chromatography method using resin, such as S-Sepharose FF (product made from Pharmacia). The hydrophobic chromatography method using resin, such as butyl sepharose and phenyl sepharose, independent in technique, such as electrophoresis methods, such as gel filtration using molecular sieving, the affinity chromatography method, the chromatofocusing method, and isoelectric focusing. — or it can combine and use and a purification preparation can be obtained.

[0106] Moreover, when this polypeptide forms an insoluble object in intracellular and is

discovered, the insoluble objects of a polypeptide are collected as a precipitate fraction by crushing after collecting cells similarly and performing centrifugal separation. The collected insoluble object of a polypeptide is solubilized with a protein modifier. After returning this polypeptide to a normal spacial configuration by diluting or dialyzing this solubilization liquid, the purification preparation of this polypeptide can be obtained according to the same isolation purification method as the above.

[0107] When derivatives, such as a polypeptide of this invention or its sugar qualification object, are secreted out of a cell, derivatives, such as this polypeptide or its sugar chain adduct, can be collected to a culture supernatant. That is, a purification preparation can be obtained by acquiring a soluble fraction and using the same isolation purification method as the above from this soluble fraction by processing this culture by technique, such as the same centrifugal separation as the above.

[0108] moreover, the polypeptide of this invention — Fmoc — law (fluorenyl methyloxy carbonyl process) and tBoc — it can manufacture also by chemosynthesis methods, such as law (t-

butyloxy carbonyl process). Moreover, chemosynthesis can also be carried out using peptide synthesis machines, such as Advanced ChemT ech, Perkin-Elmer, Amersham Pharmacia Biotech, Protein Tec hnology Instrument, Synthecell-Vega, PerSeptive, and Shimadzu.

[0109] 4. Antibodies which recognize the polypeptide of this invention, such as a polyclonal antibody and a monoclonal antibody, are producible by using as an antigen the synthetic peptide which has some amino acid sequences of the purification preparation of the partial fragment polypeptide of this invention, or this polypeptide, or the polypeptide of this invention.

[0110] (1) A polyclonal antibody is producible by medicating the inside of hypodermically [ of an animal ], and a vein, or intraperitoneal with a suitable adjuvant (for example, [ Freund's complete adjuvant (Complete Freund's Adjuvant) or aluminium hydroxide gel, a pertussis vaccine, etc.], using as an antigen the peptide which has some amino acid sequences of the overall length of the polypeptide of production this invention of a polyclonal antibody, the purification preparation of the partial fragment polypeptide of this polypeptide, or the polypeptide of this invention.

[0111] As an animal prescribed for the patient, a rabbit, a goat, the rat of three to 20 weeks old, a mouse, a hamster, etc. can be used. The dose of this antigen has desirable 50-100microper animal g. When using a peptide, it is desirable to use as an antigen what carried out covalent bond of the peptide to carrea protein, such as a SUKASHI guy hemocyanin (keyhole limpet haemocyanin) and cow thyroglobulin. The peptide used as an antigen is compoundable with a peptide synthesis machine.

[0112] Administration of this antigen is performed 3 to 10 times every one — two weeks after the 1st administration. It will collect blood from an eye grounds venous plexus after each administration on three — the 7th, and will check that this blood serum reacts with the antigen used for immunity with enzyme immunoassay [enzyme immunoassay (ELISA method):\*\*\*\*\* (1976), Antibodies-A Laboratory Manual, and Cold Spring Harbor Laboratory (1988)] etc.

[0113] The blood serum can acquire a blood serum from the nonhuman mammal which showed sufficient antibody titer to the antigen used for immunity, and a polyclonal antibody can be acquired by separating and refining this blood serum. As an approach of separating and refining, independent or the approach of combining and processing is mentioned in the chromatography using centrifugal separation, the salting-out by 40 — 50% saturation ammonium sulfate, caprylic-DEAE-sepharose [Antibodies, A Laboratory manual, and Cold Spring Harbor Laboratory (1988)] or a column etc.

[0114] (2) Offer the rat which the blood serum showed sufficient antibody titer as a source of supply of an antibody forming cell to the partial fragment polypeptide of the polypeptide of this invention used for the preparation immunity of (Production a) antibody sexuparaous cell of a monoclonal antibody. A spleen will be extracted on three — the 7th, after carrying out the last administration of the antigen matter at the rat which showed this antibody titer.

[0115] Beating of this spleen is carried out in an MEM culture medium (NISSUI PHARMACEUTICAL CO, LTD. make), and it unfolds with pincettes, and supernatant liquid is thrown away after carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes. After processing the splenic cells of the obtained precipitate fraction for 1 — 2 minutes with the tris-ammonium-chloride buffer solution (pH7.65) and removing an erythrocyte, it washes 3 times by the MEM culture medium, and the obtained splenic cells are used as an antibody forming cell.

[0116] (b) Use the established cell line acquired from the mouse or the rat as a preparation myeloma cell of a myeloma cell. For example, 8-azaguanine resistance mouse (BALB/c origin) myeloma cell stock P3-X63Ag8-U1 [Curr. Topics Microbiol. Immunol., 81, and 1 (1978), (It abbreviates to P3-U1 hereafter) Europ. J. Immunol., 6, 511 (1976)], SP2 / O-Ag14 (SP-2) [Nature, 276, and 269 (1978)], P3-X63-Ag8653 (853) [J. Immunol., 123, and 1548] (1979) P3-X63-Ag8 (X63) [Nature, 256, and 495 (1975)] etc. can be used. These cell strains to 8-azaguanine culture-medium [RPMI-1640 culture medium A glutamine (1.5 mmol/l). Although a passage is carried out by culture-medium] which added 8-azaguanine (15microg/(ml)) to the culture medium (henceforth a normal culture medium) which added 2-mercaptoethanol (5x10-5 mol/l), JIENTA mycin (10microg/(ml)), and fetal calf serum (FCS) (CSL company make, 10%) further It cultivates

by the normal culture medium three - four days before cell fusion, and these 2x10<sup>7</sup> or more cells are used for fusion.

[0117] (c) Throw away supernatant liquid after an MEM culture medium or PBS (1.83g [ of phosphoric-acid disodium ] and phosphoric-acid 1 potassium 0.21g, 7.65g of salt, 1l. of distilled water, pH7.2) is sufficient, and washing the antibody forming cell acquired by production (b) of a hybridoma, and the myeloma cell acquired by (b), mixing so that the number of cells may be set to antibody forming cell:myeloma cell =5-10:1, and carrying out at-long-intervals alignment separation by 1,200rpm for 5 minutes.

[0118] Unfolding the cell population of the obtained precipitation fraction well, and stirring to this cell population, at 37 degrees C, 0.2-1ml of solutions which mixed per 108 antibody forming cells, polyethylene-glycol-1000(PEG-1000) 2g, MEM 2ml, and dimethyl sulfoxide (DMSO) 0.7ml is added, and 1-2ml of MEM culture media is added several times for [ every ] further 1 - 2 minutes.

[0119] After addition, it prepares so that an MEM culture medium may be added and the whole quantity may be set to 50ml. Supernatant liquid is thrown away for this preparation liquid after 5-minute alignment separation at long intervals by 900rpm. After unfolding the cell of the obtained precipitate fraction gently, it depends and absorbs to a measuring pipet, and blows off and appears in it, and it is gently suspended in HAT-medium [culture medium which added hypoxanthine (10-4 mol/l), thymidine (1.5x10-5 mol/l), and aminopterin (4x10-7 mol/l) to normal culture medium] 100ml.

[0120] This suspension is poured distributively 100microl / hole every on the plate for: 96 hole culture, and it cultivates for seven - 14 days at 37 degrees C among 5% CO2 incubator. The hybridoma which reacts to the partial fragment polypeptide of the polypeptide of this invention specifically is chosen after culture with the enzyme immunoassay which takes a part of culture supernatant and is stated to anti BODIZU [Antibodies, A Laboratorymanual, Cold Spring Harbor Laboratory, and Chapter 14 (1988)] etc.

[0121] The following approaches can be mentioned as a concrete example of enzyme

immunoassay. The coat of the partial fragment polypeptide of the polypeptide of this invention used for the antigen is carried out to a suitable plate in the case of immunity. The purification antibody obtained by the hybridoma culture supernatant or the below-mentioned (d) is made to react as the first antibody. After making the anti-rat or anti-mouse immunoglobulin antibody which furthermore carried out the indicator with a biotin, an enzyme, the chemiluminescence matter, or a radiation compound as the second antibody react, the reaction according to a marker is performed. What reacts to the polypeptide of this invention specifically is chosen as a hybridoma which produces the monoclonal antibody of this invention.

[0122] The thing repeats cloning twice by limiting dilution, and [uses 1st HT culture medium (culture medium excluding aminopterin from the HAT medium), and uses the 2nd normal culture medium] and in which it was stabilized and strong antibody titer was accepted is chosen as a hybridoma stock which produces the monoclonal antibody of this invention using this hybridoma. (d) Inject intraperitoneal with the 20x10<sup>6</sup> cell / [ the monoclonal antibody production hybridoma cell 5 - ] \*\* to the polypeptide of this invention acquired by (c) to the mouse of eight to 10 weeks old or nude mouse which carried out preparation pristane processing [2, 6, 10, and 14-tetramethyl pentadecane (Pristane) 0.5ml are injected intraperitoneally, and it breeds for two weeks] of a monoclonal antibody. A hybridoma is ascites-tumor-sized in ten - 21 days.

[0123] Ascites is extracted from this ascites-tumor-sized mouse, at-long-intervals alignment separation is carried out by 3,000rpm for 5 minutes, and solid content is removed. A monoclonal antibody can be refined and acquired from the obtained supernatant liquid by the approach used by the polyclonal, and the same approach. The decision of the subclass of an antibody is made using a mouse monoclonal antibody typing kit or a rat monoclonal antibody typing kit. The amount of protein is computed from a Lowry method or the absorbance in 280nm.

[0124] 5. State the method of preparation of the recombination virus vector for producing the polypeptide of this invention in specific human tissue to below the method of preparation of the recombination virus vector which produces the polypeptide of this invention. The DNA fragment of the suitable die length which contains a code part [ polypeptide / this ] if needed based on

the perfect length cDNA of DNA of this invention is prepared.

[0125] A recombination virus vector is developed by inserting the perfect length cDNA or this DNA fragment in the lower stream of a river of the promoter in a virus vector. In the case of an RNA virus vector, a recombination virus is developed by adjusting a homologous RNA fragment to the DNA fragment of the suitable die length which contains in the perfect length cDNA of DNA of this invention the part which carries out the code of homologous cRNA or this polypeptide, and inserting them in the lower stream of a river of the promoter in a virus vector. An RNA fragment chooses one of the single strands of a sense chain or an antisense strand according to the class of virus vector besides 2 chains. For example, in the case of a Sendai Virus vector, homologous RNA is conversely chosen as an antisense strand for RNA which carries out homologous of the case of a retrovirus vector to a sense chain.

[0126] This recombination virus vector is introduced into the packaging cell which suited this vector. All the cells that can supply the polypeptide to which the recombination virus vector which is missing in at least one of the DNA which carries out the code of the polypeptide which needs a packaging cell for PAKKEJI-NGU of a virus this suffers a loss can be used, for example, can use HEK293 cell of the Homo sapiens kidney origin, mouse fibrocyte NIH3 T3, etc. As a polypeptide supplied in a packaging cell In the case of a retrovirus vector, gag of the mouse retrovirus origin, In the case of a lentivirus vector, polypeptides, such as pol and env, gag of the HIV origin, Polypeptides, such as pol, env, vpr, vpu, vif, tat, rev, and nef, In the case of an adenovirus vector, polypeptides, such as E1A of the adenovirus origin and E1B In the case of an adeno-associated virus, polypeptides, such as Rep (p5, p19, p40) and \*\*\*\* (Cap), are mentioned, and in the case of Sendai Virus, polypeptides, such as NP, P/C, and L, M, F, HN, are mentioned. [0127] As a virus vector, it rearranges in the above-mentioned packaging cell, a virus can be produced, and the thing containing a promoter is used for the location which can imprint DNA of this invention by the target cell. As a plasmid vector, MFG [Proc.Natl.Acad.Sci.USA, 92, and 6733-6737 (19 95)], pBabePuro [Nucleic Acids Res., 18, and 3587-3596 (1990)], LL-CG, CL-CG, CS-CG, and CLG [Journal of Virology, 72, and 8150-8157 (1998)], pAdex1 [Nucleic Acids Res., 23, and 3816-3821 (1995)] etc. is used.

[0128] As a promoter, if it can be discovered all over human tissue, all can be used, for example, the promoter of IE (immediateearly) gene of a cytomegalovirus (Homo sapiens CMV), the initial promoter of SV40, the promoter of a retrovirus, a metallothionein promoter, a heat shock protein promoter, SRalpha promoter, etc. can be mentioned. Moreover, the enhancer of Homo sapiens s CMV IE gene may be used with a promoter.

[0129] As a method of introducing the recombination virus vector to a packaging cell, a calcium phosphate method [JP 2-227075A], the RIPOFE cushion method [Proc.Natl.Acad.Sci.U SA, 84, and 7413 (1987)], etc. can be mentioned, for example.

6. A structural change of the amount of mRNA manifestations of DNA of this invention in a specimen and this mRNA is detectable using DNA of approach this invention which detects the manifestation of DNA of use (1) this invention of DNA of this invention, a polypeptide, or an antibody.

[0130] The organization which acquired from the patient and healthy person who have as a specimen the disease from which manifestation change of DNA of this invention is the cause. Biological materials, such as a blood serum and saliva, the primary culture cell sample which acquired the cell from this biological material and was cultivated in the suitable culture medium in a test tube, Or mRNA or all RNA acquired from what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept is used (this mRNA and all RNA are henceforth called the specimen origin RNA).

[0131] As an approach of detecting, approaches, such as a (1) Northern-blot-technique (2) in situ hybridization method, (3) quantitative PCR method, (4) differential hybridization method [Trends in Genetics 7 and 314 (1991)], (5) DNA-chip method [Genome Research, 6, and 639 (1996)], and the (6) RNase protection assay method, etc. are mentioned, for example. Hereafter, each detecting method is explained in full detail.

[0132] \*\* Imprint the Northern blot technique specimen origin RNA to base materials, such as a nylon filter, after separation by gel electrophoresis. Hybridization and washing are performed

after an imprint using the indicator probe prepared from DNA of this invention. The band of RNA specifically combined with this probe is detected after washing. By comparing this detection result with a healthy person about the specimen RNA of the patient origin, the amount of manifestations of this RNA and change of structure are detectable. In case hybridization is performed, mRNA made into the purpose under a probe and specimen origin RNA carries out an incubation on the conditions which form a stable hybrid, the approach of an edition [ of molecular cloning / 2nd ] publication of hybridization and a washing process in order to prevent false positivity --- applying correspondingly --- quantity --- it is desirable to carry out on stringent conditions.

[0133] The indicator probe used for a Northern blot technique can be prepared by making the oligonucleotide which designed the radioisotope, the biotin, the fluorescence radical, the chemiluminescence radical, etc. from the array of DNA of this invention, or this DNA by the well-known approach (nick translation, a random priming, or KINAJINGU), for example incorporate. The amount of association to mRNA of an indicator probe can carry out the quantum of the amount of manifestations of this mRNA by carrying out the quantum of the amount of the united indicator probe from reflecting the amount of manifestations of this mRNA. Moreover, a structural change of this mRNA can be known by analyzing the part on the filter which an indicator probe combines.

[0134] \*\*in Perform hybridization and the process of washing using the specimen which isolated the organization which acquired from the situ hybridization method living body as paraffin or a cryostat intercept, and was obtained, and an indicator probe given in \*\*. The amount of manifestations of mRNA specifically combined with this probe by the same approach as \*\* is detectable after washing. in the approach indicated by current PUOTO call Inn molecular biology etc. in hybridization and a washing process by the situ hybridization method in order to prevent false positivity --- applying correspondingly --- quantity --- it is desirable to carry out on stringent conditions.

[0135] \*\* Target RNA is detectable by using the approach based on compounding cDNA using the quantitative PCR method specimen origin RNA, an oligo dT primer or a random primer, and reverse transcriptase (this cDNA is henceforth called the specimen origin cDNA). When the specimen origin RNA is mRNA, any primer of the above-mentioned \*\* can be used, but when these specimen origins RNA are all RNA, it is required to use an oligo dT primer.

[0136] At the quantitative PCR method, the DNA fragment of the specific mRNA origin is amplified by performing PCR using the primer designed based on the base sequence which makes the specimen origin cDNA a template and DNA of this invention has. Since the amount of this magnification DNA fragment reflects the amount of manifestations of this mRNA, it can carry out the quantum of the amount of this mRNA by placing DNA which carries out the code of an actin, G3 PDH (glyceraldehyde 3-phosphate dehydrogenase), etc. as internal control. Moreover, change of the structure of this mRNA can also be known by separating this magnification DNA fragment by gel electrophoresis. It is desirable to use the suitable primer which amplifies a target sequence specifically and efficiently by this detecting method. Neither association between primers nor association in a primer can be caused, but it can combine with Target cDNA specifically at annealing temperature, and a suitable primer can be designed based on conditions, such as shifting, from Target cDNA on denaturation conditions. The quantum of a magnification DNA fragment needs to carry out to the inside of the PCR reaction which the magnification product is increasing exponentially. Such an PCR reaction can be known by collecting these magnification DNA fragments produced for every reaction, and carrying out quantitative analysis by gel electrophoresis.

[0137] \*\* Perform hybridization and washing to the base of the filter or slide glass which made DNA of this invention fix, silicon, etc. by using as a probe the specimen origin cDNA prepared by the approach indicated by differential hybridization method and DNA chip method \*\*. Fluctuation of the amount of manifestations of mRNA of this cDNA origin is detectable after washing by measuring the amount of cDNA(s) specifically combined with DNA of this invention. The difference in the manifestation of this mRNA between a contrast specimen and a target specimen is correctly detectable because any approach of a differential hybridization method and

a DNA chip method fixes internal control of an actin, G3 PDH, etc. on a filter or a base. Moreover, indicator cDNA composition can be performed using an indicator dNTP different, respectively based on a contrast specimen and RNA of the target specimen origin, and the quantum of the amount of manifestations of this exact mRNA can be performed by making the filter of one sheet, or the base of one sheet hybridize two indicator cDNA probes to coincidence.

[0138] \*\* Combine promotor arrays, such as T7 promotor and SP6 promotor, with 3' edge of DNA of RNase protection assay method this invention, and compound the antisense RNA which carried out the indicator using rNTP which carried out the indicator by the imprint system of in vitro using RNA polymerase. After combining this indicator antisense RNA with the specimen origin RNA and making a RNA-RNA hybrid form, it digests by RNase, and a band is made to form by gel electrophoresis and the RNA fragment protected from digestion is detected. By carrying out the quantum of the obtained band, the quantum of the amount of manifestations of mRNA combined with the above-mentioned indicator antisense RNA can be carried out.

[0139] In addition, the DNA fragment obtained from DNA or them which have the base sequence expressed with either of the array numbers 6-10, for example as DNA used for the approach indicated to either \*\* - \*\* is mentioned, moreover, as a specimen with which detection by the approach concerned is presented The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis, The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue. Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome), Diseases, such as adult respiratory distress syndrome (ARDS:adultrespiratory distress syndrome), are mentioned, and it can use for a diagnosis of the above-mentioned disease by detecting the manifestation of DNA of this invention by the detection approach concerned. [0140] (2) Describe how to detect the existence of the variation of DNA of this invention in a test subject, below the approach of detecting the variation of DNA of this invention. The variation of this DNA in a test subject is detectable by comparing directly by DNA and the following approach of this invention. From a test subject, the samples of the primary culture cell origin established from a Homo sapiens biological material or these biological materials, such as an organization, a blood serum, and saliva, are collected, and DNA is extracted out of this biological material or this primary culture cell origin sample (this DNA is hereafter called the specimen origin DNA). Or cDNA is acquired from mRNA of this sample origin with a conventional method (this cDNA is hereafter called the specimen origin cDNA). These specimen origins DNA and cDNA are used as mold, and DNA is amplified by the PCR method etc. using the primer designed based on the base sequence which DNA of this invention has. The obtained magnification DNA is used as a sample DNA.

[0141] The approach of detecting the heteroduplex formed as an approach of detecting whether variation being in Magnification DNA, of hybridization with the DNA strand which has a wild type allele, and the DNA strand which has variation allele can be used. The heteroduplex detecting method according to \*\* polycrylamide gel electrophoresis in the approach of detecting a heteroduplex [Trends Genet., 7, and 5 (1991)]. \*\* A single strand conformation polymorphism analysis method [Genomics, 16, and 325-332 (1993)]. \*\* Chemical cleavage method (CCM, chemical cleavage of mismatches) [Human Molecular Genetics (1996) of a mismatch, Tom

Strachan and Andre w P Read (BIOS Scientific Publishers Li mited)]. \*\* The enzyme-intercept method of a mismatch [Nature Genetics, 9, and 103-104 (1996)]. \*\* Denaturation gel-electrophoresis [Mutat Res., The approach of 288, a 103-112 (1993)]\*\* protein compaction trial (the protein truncation test:PTT method) [Genomics, 20, and 1-4 (1994)]. etc. is mentioned. Hereafter, the above-mentioned approach is explained.

[0142] \*\* Amplify as a DNA fragment smaller than 200bp by the primer which designed the heteroduplex detecting method specimen origin DNA by polyacrylamide gel electrophoresis, or the specimen origin cDNA to the template based on the base sequence given [ this DNA ] in either of the array numbers 6-10. 2 chain formation processing by each magnification DNA fragment is performed with a conventional method using DNA of this invention, and this magnification DNA fragment of the test subject origin. Polyacrylamide gel electrophoresis is magnified after processing. When a heteroduplex is formed of the variation of this DNA, mobility is later than a gay double strand without variation, and they can be detected as a band different from a gay double strand. It is better for degree of separation to use gels (Hydro-link, MDE, etc.) of special make. If it is retrieval of a fragment smaller than 200bp(s), insertion, deletion, and almost all 1 base substitution are detectable. As for heteroduplex analysis, it is desirable to carry out by the gel of one sheet combined with the single strand conformation polymorphism analysis described below.

[0143] \*\* Carry out electrophoresis of this DNA amplified as a fragment smaller than 200bp in native polyacrylamide gel after denaturalizing by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication in single strand conformation polymorphism analysis-method single strand conformation polymorphism analysis (SSCP analysis; single strand conformation polymorphism analysis). This amplified DNA is detectable as a band by carrying out the indicator of the primer by radioisotope or the fluorochrome, in case DNA magnification is performed, making this indicator into an index, or carrying out the argentation of the magnification product of a non-indicator after electrophoresis. A fragment with variation is detectable from the difference in mobility by carrying out electrophoresis of the magnification DNA fragment of the DNA origin of this invention, and the thing of the test subject origin to coincidence.

[0144] \*\* In the chemical cleavage method (the CCM method) of the chemical cleavage method making DNA of a mismatch, one chain of DNA of the location which is carrying out the mismatch by making DNA of this invention hybridize the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template based on the base sequence given [ this DNA ] in either of the array numbers 6-10 with the indicator DNA which made the radioisotope or the fluorochrome take in, and processing it with an osmium tetroxide can be made to be able to cut, and variation can be detected. The CCM method is one of the detecting methods sensibility is the highest, and can be adapted also for the specimen of the die length of kilobase.

[0145] \*\* A mismatch can also be cut in [ combining with the T4 phage RIZORU base, the enzyme which participates in restoration of a mismatch by intracellular / like Endonuclease VII /, and RNaseA ] enzyme instead of the enzyme-intercept method above-mentioned osmium tetroxide of a mismatch.

\*\* Carry out electrophoresis of the DNA fragment amplified by the primer which designed the specimen origin DNA or the specimen origin cDNA to the template at either of the array numbers 6-10 based on the base sequence of a publication using the gel which has the concentration gradient and temperature gradient of a chemical modifier in denaturation gel-electrophoresis denaturation gel electrophoresis (denaturing gradient gel electrophoresis:DGGE law). The amplified DNA fragment will move in the inside of gel to the location which denaturalizes to a single strand, and after denaturation will not move it. Since the mobility within the gel of DNA amplified in the case where there is nothing with the case where variation is in this DNA differs, it is possible to detect existence of variation. It is good to attach a Pori (G:C) terminal for raising detection sensitivity at each primer.

[0146] \*\* Protein compaction trial (the protein truncation test:PTT method)

The phase shift mutation which produces the deficit of a polypeptide by this trial, splice site

mutation, and nonsense mutation are specifically detectable, the special primer which connected T7 promotor array and the eukaryote translational initiation sequence with the five prime end of DNA which has the base sequence expressed with the PTT method to either of the array numbers 6-10 --- designing --- this primer --- using --- the specimen origin RNA --- reverse transcription PCR (RT-PCR) --- cDNA is created by law. A polypeptide will be produced if an in vitro imprint and a translation are performed using this cDNA. When this polypeptide is migrated to gel, the variation which produces a deficit does not exist if it is in the location where the migration location of this polypeptide is equivalent to a perfect length polypeptide, but a deficit is in this polypeptide, this polypeptide can migrate in a location shorter than a perfect length polypeptide, and extent of a deficit can be known from this location.

[0147] When variation is detected by the above-mentioned approach, it is possible to determine the base sequence of the specimen origin DNA which has variation with a conventional method, and the specimen origin cDNA using the primer designed based on the base sequence which DNA of this invention has. In the case of the test subject in whom the specimen origin DNA or the specimen origin cDNA has a specific disease, the variation leading to this disease can be specified by analyzing the determined base sequence. Henceforth, it can use for a diagnosis of a disease by detecting this variation.

[0148] In detection of variation other than the variation in the coding region of DNA detected by the above-mentioned approach, it can detect by inspecting the intron near this DNA and in this DNA, and a non-coding region like a regulatory sequence. The disease resulting from the variation in a non-coding region can be checked by detecting the unusual size in the disease patient at the time of comparing with a contrast specimen according to the approach indicated above, or mRNA of an unusual volume.

[0149] Thus, about this DNA existence of the variation in a non-coding region was suggested saying, it can clone by using for either of the array numbers 6-10 DNA which has the base sequence of a publication as a probe of hybridization. It can search for the variation in a non-coding region according to one of above-mentioned approaches.

[0150] The found-out variation can be identified as SNPs (single nucleotide poly mol FIZUMU) with a chain with a disease by performing statistics processing according to the approach indicated by Handbook of Human Genetics Linkage. The John Hop kins University Press and Baltimore (1994). As a diagnosable test subject, by the approach of detecting the above-mentioned variation The disease accompanied by activation of unusual immunocytes, such as allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, psoriasis, gout, various encephalomyelitis. The disease accompanied by infection and inflammation of congestive heart failure, traumatic brain injury, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, articular rheumatism, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemicinflammatory response syndrome). Those who have ones, such as adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome), of diseases can be mentioned.

[0151] (3) The approach antisense RNA / DNA technical [bioscience and the industry which control the imprint or translation of DNA which carries out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention, and 50:322 (1992). Chemistry, 46, 681 (1991). Biotechnology, 9, and 358 (1992). Trends in Biotechnology, 10, and 87 (1992). Trends in Biotechnology, 10, and 152 (1992). With a cell technology, 16, 1463 (1997)]. a triple helix technique [Trends in Biotechnology, 10, and 132 (1992)]. etc. The imprint or translation of DNA



which carries out the code of the polypeptide of this invention can be controlled using DNA of this invention. For example, the system (a living body is included) which can discover the polypeptide of this invention for DNA or the oligonucleotide of this invention is made to live together, and the manifestation of this polypeptide can be controlled on an imprint and translation level.

[0152] This control approach Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease, The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, psoriasis, gout, various encephalomyelitis, congestive heart failure, traumatic brain injury. The disease accompanied by infection and inflammation of inflammatory bowel disease etc., a Burkitt lymphoma, Hodgkin's disease, The disease accompanied by unusual cell proliferations, such as various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as articular rheumatism and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy. The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic in inflammatory response syndrome), The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for the therapy or prevention of a disease used as a cause.

[0153] (4) It is possible to acquire the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention by the well-known approach [the volume the 2nd edition of molecular cloning and for University of Tokyo Institute of Medical Science carcinostatic research sections, a new cell technology experiment protocol, and Shujunsha (1993)], using as a probe DNA or the oligonucleotide of approach this invention which acquires the promoterregion and the imprint regulatory region of DNA which carry out the code of the polypeptide of this invention using DNA or the oligonucleotide of this invention. For example, the thing of a rat or the Homo sapiens origin is acquirable by the following approaches. [0154] It screens by approaches, such as plaque hybridization, to the genomic DNA library produced using the chromosome DNA isolated from cell and organization of a rat or Homo sapiens by using DNA or the oligonucleotide (especially 5' of cDNA near part) of this invention as a probe. The genomic DNA to hybridize is acquired by this screening. Promoterregion and imprint regulatory region can be obtained from this DNA. Moreover, an exon / intron structure can be clarified by comparing the base sequence of genomic DNA and the base sequence of cDNA which were acquired.

[0155] In addition, also in other nonhuman mammals, the promoterregion and the imprint regulatory region of this DNA are acquirable using the same approach. The field which participates in the basic imprint of DNA which carries out the code of the polypeptide of this invention in a mammalian cell as promoterregion is mentioned, and a field including an enhancer sequence, a silencer array which decreases which reinforces the basic imprint of DNA which carries out the code of the polypeptide of this invention as imprint regulatory region is mentioned. For example, the promoterregion and the imprint regulatory region which participate in the imprint of DNA which carries out the code of the polypeptide of this invention by human bone marrow can be mentioned. The promoter and imprint regulatory region which were obtained are applicable to the below-mentioned screening approach, and also they are useful in order to analyze the controlling mechanism of an imprint of this DNA.

[0156] (5) Various test compounds can be added to the cell strain of the approach patient origin which acquires the physic which controls the imprint of this DNA by screening using DNA which carries out the code of the polypeptide of this invention, and the matter which controls or promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of mRNA using DNA of this invention. The change in the manifestation of mRNA of

this DNA is detectable by the above-mentioned PCR method and the above-mentioned Northern blot technique, and the RNase protection assay method.

[0157] Various test compounds can be added to a patient origin cell strain, and the matter which promotes an imprint or translation of this DNA can be screened by authorizing the change in the manifestation of this polypeptide using the antibody which recognizes the polypeptide of this invention specifically. The change in the manifestation of this polypeptide is detectable by immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, the western blotting method, the dot blotting method, the immunoprecipitation method, and the sandwiches ELISA method.

[0158] The polypeptide of this invention on moreover, the lower stream of a river of the promoter region of DNA which carries out a code, and imprint regulatory region The reporter plasmid which connected the chloramphenicol acetyltransferase (CAT) gene and the luciferase gene as a reporter gene is built. After introducing into a suitable cell host and obtaining a transformant, the physic which controls by imprint level the manifestation of DNA which carries out the code of the polypeptide of this invention can be screened by adding various examined substances to the transformant, and analyzing the change in the manifestation of a reporter gene.

[0159] (6) How to acquire the physic which acts on the polypeptide of this invention by the screening approach using the polypeptide of this invention.

The physic which acts on the polypeptide of this invention can be screened by making the transformant which discovered the polypeptide of this invention, or the partial peptide of this polypeptide, and various examined substances live together, and analyzing fluctuation of activation of NF-kappa B in this transformant. Moreover, it can use for the medicinal screening to which the partial peptide of this refined polypeptide or this polypeptide also acts on this polypeptide specifically. The matter obtained by this screening is useful as physic for the therapy of the disease in which DNA and the polypeptide of this invention participated.

[0160] Hereafter, two sorts of screening procedures are explained.

#### Screening procedure (1)

The microorganism which carried out the transformation so that the polypeptide of this invention or the partial peptide of this polypeptide might be produced, an animal cell or an insect cell (the transformant for retrieval is called henceforth), and an examined substance are made to live together in an aqosity medium. According to the approach of a publication, the activity of NF-kappa B is measured after coexistence to above-mentioned 2. Microorganism, animal cell, or insect cell of the host who has not done a transformation is compared as a control group, and the target matter can be acquired by choosing the examined substance which fluctuates extent of activation of NF-kappa B in this transformant. Moreover, it can make into an index to check association to this transformant for retrieval of the compound specifically combined with this transformant for retrieval, or a polypeptide, and contention screening of the target compound can be carried out by the same approach as the above.

[0161] The polypeptide which constitutes a part of polypeptide of refined this invention or this polypeptide can be used for choosing the target compound specifically combined with this polypeptide. In order to carry out the quantum of the target compound, the polypeptide of this invention can be performed by the above-mentioned immunologic procedure using the antibody recognized specifically. Moreover, contention screening of the target compound can be carried out for checking association of the target compound combined with the polypeptide of this polypeptide or this polypeptide at an index.

#### [0162] Screening procedure (2)

Many peptides which constitute this a part of polypeptide can be compounded to high density on a plastics pin or a solid-state base material of a certain kind, and the compound or polypeptide alternatively combined with this peptide can be screened efficiently (WO 84/03564). In addition, the gene which receives transcriptional control by the polypeptide of this invention can be screened by analyzing gene expression using the transformant which discovers the polypeptide

of this invention.

[0163] (7) The gene therapy agent using the virus vector containing RNA which consists of DNA of this invention, DNA of gene therapy agent this invention containing RNA which consists of this DNA and a homologous array or this DNA, and a homologous array can be manufactured by preparing the basis which was produced by above-mentioned 5, and which is rearranged and is used for a virus vector and a gene therapy agent [Nat ure Genet., 8, and 42 (1994)]. If it is the basis usually used for injections as a basis used for a gene therapy agent, what kind of thing may be used and the mixed solution of amino acid solutions, such as sugar solutions, such as salting in liquid, such as mixture of distilled water, a sodium chloride or a sodium chloride, and mineral salt, a mannitol, a lactose, a dextran, and a glucose, and a glycine, and an arginine, an organic-acid solution or salting in liquid, and a glucose solution etc. will be raised. Moreover, according to a conventional method, assistants, such as surfactants, such as vegetable oil, such as an osmotic-pressure regulator, pH regulator, sesame oil, and soybean oil, lecithin, or a nonionic surface active agent, may be used for these bases, and injections may be prepared as a solution, suspension, and dispersion liquid. these injections -- actuation of disintegration, freeze drying, etc. -- business -- the time -- as the pharmaceutical preparation for the dissolution -- it can also prepare. In the case of a liquid, the gene therapy agent of this invention remains as it is, and in the case of an individual, it can dissolve in the above-mentioned basis which carried out sterilization processing as occasion demands just before gene therapy, and can be used for a therapy. As a medication method of the gene therapy agent of this invention, the approach of prescribing for the patient locally can be raised so that it may be absorbed by a patient's therapy part.

[0164] A virus vector can be prepared by combining with an adenovirus vector the complex which produced complex combining the specific poly lysine-conjugate antibody in adenovirus hexone protein, and was obtained in DNA of suitable this invention of size. Stability is reached at a target cell, and it is incorporated by intracellular by endosome, and is decomposed by intracellular, and this virus vector can make DNA discover efficiently.

[0165] (-) The virus vector which used as the base Sendai Virus which is a chain RNA virus is also developed (Japanese Patent Application No. 9-517213, Japanese Patent Application No. 9-517214), and the Sendai Virus vector which incorporated KRGF-1 gene for the purpose of gene therapy can be produced. This DNA can be conveyed to the focus also by the non-virogene importing method.

[0166] By the well-known non-virogene importing method, in the field concerned A calcium phosphate coprecipitation method [Virology, 52, 456-467;(1973) Science, 209, and 1414-1422 (1980)], Microinjection method [Proc. Natl.Acad.Sci.USA, 77 and 5399-5403 1980 .P roc.Natl.Acad.Sci.USA, 77, 7380-7384;(1980) Cell, 27, 223-231;(1981) Nature, 294, and 92-94 (1981) -- ] -- Liposome Minded membrane fusion--mediation importing method [Proc.Natl. Acad.Sci.USA and 84, 7413-7417;(1987) Biochemistry, 28, 9508-9514;(1989) J.Biol.Chem., 264, and 12126-12129;(1989) Hum. Gene T her. and 3,267-275 () 1992;Science and 249, Method [ of 1285-1288;(1990) Circulation, 83 2007-2011 (1992)] or direct DNA incorporating, and acceptor-medium DNA importing [ Science, 247, and 1485-1468 .J.Biol.Chem., (1990) 266 14338-14342 (1991) .P roc.Natl.Acad.Sci.USA, 87, 3655-3659;(1991) J.Biol.Chem., 26 4 and 16985-16987 ; BioTechniques, (1989) 11 474-485 (1991) .P roc. Natl.Acad.Sci.USA, 87 3410-3414 (1990) .P roc. Natl.Acad.Sci.USA, 88 4255-4259 (1991) .P roc. Natl.Acad.Sci.USA, 87 4033-4037 (1990) .P roc.Natl.Acad.Sci.USA, 88, 8850-8854;(1991) Hum. Gene Ther., 3, 147-154(1991)], etc. can be mentioned.

[0167] By the membrane fusion--mediation importing method through liposome, it is reported in the research on a neoplasm by medicating with a liposome preparation object directly the organization which considers as a target that incorporation and manifestation of the organization concerned of a local gene are possible [Hum. Gene Ther., 3, and 399-410 (1992)]. Therefore, the same effectiveness is expected also by the disease focus in which DNA and the polypeptide of this invention participate. In order to carry out direct targeting of the DNA to the focus, a direct -DNA incorporation technique is desirable. Acceptor-medium DNA import is performed for example, through the poly lysine by carrying out conjugate of the DNA (the gestalt of the

supercoiling plasmid which usually carried out the ring closure in share being taken) to polypeptide ligand. Ligand is chosen based on existence of the ligand acceptor to which it corresponds on a target cell or the cell surface of an organization. By request, a blood vessel can be directly injected with the ligand-DNA conjugate concerned, and it can point to it in the target tissue to which internalization of acceptor association and DNA-protein complex takes place. In order to prevent intracellular destruction of DNA, concurrent infection of the adenovirus can be carried out and an endosome function can also be collapsed.

[0168] (8) The organization containing the polypeptide or this polypeptide of this invention is immunologically detectable by making an antigen-antibody reaction perform using the antibody which recognizes specifically the polypeptide of approach this invention which detects the polypeptide of this invention immunologically using the antibody of this invention. This detecting method Allergy, atopy, asthma, pollinosis, respiratory tract irritation, an autoimmune disease. The disease, endotoxin shock accompanied by activation of unusual immunocytes, such as graft versus host disease, Septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, an insulin dependency and non-dependency diabetes mellitus, Glomerulonephritis, traumatic brain injury, hypertrophic arthritis, psoriasis, gout, various encephalomyelitis. The disease, Burkitt lymphoma accompanied by infection and inflammation of congestive heart failure, inflammatory bowel disease, etc.. The disease accompanied by unusual cell proliferations, such as Hodgkin's disease, various lymphomas, adult T-cell leukemia, and a malignant tumor, Unusual fibroblasts, such as rheumatoid arthritis and fibroid lung, and the disease accompanied by activation of synovial membrane tissue. Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy. The disease based on the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease. The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome). The variation of DNA which carries out the code of the polypeptide of this invention can use adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc. for a diagnosis of the disease used as a cause. Moreover, this detection approach is used also for the quantum of a polypeptide.

[0169] as detection and an approach of carrying out a quantum, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as a fluorescent antibody technique, enzyme immunoassay (the ELISA method), radioactive substance indicator immuno antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method [a monoclonal antibody experiment manual (Kodansha -- scientific) (1987), New Biochemistry Experiment Lectures 5, and an immunobiochemistry approach (Tokyo Kagaku Dojin) (1986)], etc. are mentioned immunologically.

[0170] After a fluorescent antibody technique makes the antibody of this invention react to the microorganism, the animal cell, insect cell, or organization which discovered the polypeptide of this invention out of intracellular or a cell and makes the anti-mouse IgG antibody which carried out the label with fluorescent materials, such as fluorescein isothiocyanate (FITC), further, or its fragment react, it is the approach of measuring a fluorescence with flow cytometer.

[0171] Enzyme immunoassay (the ELISA method) is the approach of measuring coloring matter with an absorptiometer, after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it enzyme labeling, such as a peroxidase and a biotin, etc. further, or a joint fragment react.

[0172] Radioactive substance indicator immuno antibody technique (RIA) is the approach of measuring with a scintillation counter etc., after making the anti-mouse IgG antibody which the antibody of this invention was made to react to the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out of intracellular or a cell, and gave it the radiation indicator further, or its fragment react. After an immunocyte staining technique and an immunity staining method make the antibody which recognizes this polypeptide specifically in the microorganism, the animal cell, insect cell, or organization which discovered this polypeptide out

of intracellular or a cell react and make the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescein materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, they are the approach of observing using a microscope.

[0173] The microorganism which discovered this polypeptide out of intracellular or a cell with the western blotting method. After carrying out fractionation of an animal cell, an insect cell, or the extract of an organization by SDS-polyacrylamide gel electrophoresis [Antibodies-A Laboratory Manual and Cold Spring Harbor Laboratory (1988)]. Blotting of this gel is carried out to the PVDF film or a nitrocellulose membrane. After making the antibody which recognizes this polypeptide of this invention specifically react to this film and making the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescein materials, such as FITC, a peroxidase, and a biotin, further, or its fragment react, it is the approach of checking.

[0174] After the dot blotting method carries out blotting of the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization to a nitrocellulose membrane, makes the antibody of this invention react to this film and makes the anti-mouse IgG antibody which gave enzyme labeling, such as fluorescein materials, such as FITC, a peroxidase, and a biotin, further, or a joint fragment react, it is the approach of checking.

[0175] An immunoprecipitation method is an approach of adding the support, which has a specific binding affinity to immunoglobulins, such as protein G-sepharose, and making an antigen antibody complex sedimenting, after making the microorganism which discovered the polypeptide of this invention out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react with the antibody which recognizes this polypeptide specifically.

[0176] The sandwiches ELISA method is the antibody which recognizes the polypeptide of this invention specifically. The antibody which is one side beforehand among two kinds of antibodies from which an antigen recognition site differs is made to stick to a plate. The indicator of another antibody is carried out with enzymes, such as fluorescent materials, such as FITC, a peroxidase, and a biotin. After making the microorganism which discovered this polypeptide out of intracellular or a cell, an animal cell, an insect cell, or the extract of an organization react to an antibody adsorption plate, it is the approach of making the antibody which carried out the indicator reacting and performing the reaction according to a marker.

[0177] (9) It is useful to identify a structural change of the polypeptide which has changed and discovered the amount of manifestations of this polypeptide in the approach Homo sapiens biological material row Homo sapiens primary culture cell which diagnoses a disease using the antibody which recognizes the polypeptide of this invention specifically, when getting to know the danger of showing the symptoms of a disease in the future, and the cause of a disease whose symptoms were already shown. As an approach of detecting and diagnosing the amount of manifestations of this polypeptide, and a structural change, immunohistochemistry staining techniques (the ABC method, the CSA method, etc.), such as the above-mentioned fluorescent antibody technique and the above-mentioned enzyme immunoassay (the ELISA method), radioactive substance indicator immunity antibody technique (RIA), an immunity staining method, and an immunocyte staining technique, a western blotting method, the dot blotting method, an immunoprecipitation method, the sandwiches ELISA method, etc. are mentioned.

[0178] As a specimen with which the diagnosis by the above-mentioned approach is presented, allergy, atopy. The disease accompanied by activation of unusual immunocytes, such as asthma, pollinosis, respiratory tract irritation, an autoimmune disease, and graft versus host disease. The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, hypertrophic arthritis. The disease accompanied by infection and inflammation of psoriasis, gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia. Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations such as a malignant tumor, rheumatoid arthritis, and fibroid lung, and the disease accompanied by activation of synovial membrane tissue. Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy. The disease based on

the failure of nerve cells, such as an Alzheimer disease and Parkinson's disease. The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis. A systemic inflammatory response syndrome (SIRS:systemic inflammatory response syndrome). Adult respiratory distress syndrome (ARDS:adult respiratory distress syndrome) etc.. The cell and cell extract which were acquired from the biological material itself or these biological materials, such as the organization and blood which were acquired from the patient of the disease from which the variation of DNA which carries out the code of the polypeptide of this invention is the cause, a blood serum, urine, facilities, and saliva, are used. Moreover, what isolated the organization which acquired from the biological material as paraffin or a cryostat intercept can also be used.

[0179] The ELISA method and a fluorescent antibody technique using a microtiter plate as an approach of detecting immunologically, a Western blot technique, an immunity staining method, etc. are mentioned. The radioimmunoassay method using the antibody which recognizes the polypeptide of this invention and the polypeptide of this invention which carried out the indicator with radioisotopes using two kinds of monoclonal antibodies from which an epitope differs in the liquid phase as an approach of carrying out a quantum immunologically among the polypeptide of this invention and the antibody which reacts, such as the sandwiches ELISA method and 125I, etc. is mentioned.

[0180] (10) Use the recombination vector which comes to contain DNA of production this invention of a knock out nonhuman animal using DNA of this invention. In embryonic stem cells (embryonic stem cell), such as the target nonhuman animal, for example, a cow, a sheep, a goat, Buta, a horse, a mouse, and a fowl DNA which carries out the code of the polypeptide of this invention on a chromosome -- the technique of well-known homologous recombination -- [ -- for example, ] The chimera individual which consists of an embryonic stem cell clone and a normal cell can be prepared using the variation clone of an embryonic stem cell by technique, such as Nature, 326, 295 (1987), Cell, 51, and 503, is produced ([Nature, 350, and 243] (1991)). [ for example, ] The chimera individual which consists of an embryonic stem cell clone and a normal cell can be prepared using the variation clone of an embryonic stem cell by technique, such as the impregnation chimera method to the blastocyst (blastocyst) of the fertilized egg of an animal, or the set chimera method. The individual which has the variation of arbitration by crossing of this chimera individual and a normal individual in DNA which carries out the code of the polypeptide of this invention on the chromosome of the cell of the whole body can be obtained, and the manifestation of DNA which carries out the code of the polypeptide of this invention can obtain a knock out nonhuman animal as a part or an individual controlled completely out of the gay individual by which variation went into the both sides of homologue by crossing of that individual further.

[0181] Moreover, it is also possible to produce a knock out nonhuman animal by introducing variation to the location of the arbitration of DNA which carries out the code of the polypeptide of this invention on a chromosome. For example, it is possible to also make the activity of the product change by a permutation, deletion, insertion, etc. carrying out a base all over the translation field of DNA which carries out the code of the polypeptide of this invention on a chromosome, and introducing variation. Moreover, it is possible by introducing the same variation to the manifestation regulatory region to also make extent of a manifestation, a stage, tissue specificity, etc. change. It is also still more possible to control a manifestation stage, a manifestation part, the amount of manifestations, etc. by combination with a Cre-loxP system more positively, the example [Cell, 87, and 131 7 (1996)] to which deletion of the purpose gene was carried out only in the field using the promoter discovered in a specific field with a brain as such an example, and the adenovirus which discovers Cre -- using -- the target stage -- an organ -- the example [Science, 278, and 5335 (1997)] to which deletion of the purpose gene was carried out specifically is known.

[0182] Therefore, the knock out nonhuman animal which can control a manifestation by the stage and organization of arbitration, or has insertion of arbitration, deletion, and a permutation in the translation field and manifestation regulatory region in this way also about DNA which carries out the code of the polypeptide of this invention on a chromosome is producible. A knock out nonhuman animal can guide the symptom of the various diseases resulting from the



polypeptide of this invention by the stage of arbitration, extent of arbitration, or the part of arbitration. Thus, the knock out nonhuman animal of this invention serves as very useful animal model in the therapy and prevention of various diseases resulting from the polypeptide of this invention. It is very useful especially as models for evaluation, such as the remedy, a prophylactic and functional food, and health food.

[0183] 7. As an approach of introducing variation into the variation installation this polypeptide of the polypeptide of variation installation of the polypeptide of this invention, and selection (1) this invention of a functional alteration variant, what kind of approach of deletion, insertion, and a permutation may be used. The deletion and insertion of a polypeptide are possible by carrying out deletion of this DNA fragment by the approach indicated by the 2nd edition of molecular cloning, current PUROTO call Inn molecular biology, etc. in DNA which carries out the code of this polypeptide, or making a suitable DNA fragment insert.

[0184] For example, it can obtain by graduating by DNA polymerase, such as Klenow Fragment (product made from TaKaRa), and making it re-connect after digestion, with this restriction enzyme of marketing of the plasmid which included a the same and different restriction enzyme site suitable in this DNA for a two-piece header and this DNA when it was a deletion mutant, if it is a flush end, it is a cohesive end as it is. If it is an insertion variant, it can obtain by making double stranded DNA suitable after flush-end-izing insert and connect. A permutation variant is Error Prone as an approach of introducing variation at random. The PCR method [Trends In Biotechnology, 16, and 76 (1998)] etc. can be used. As an approach of introducing variation into the target location, the PCR method [Mutagenesis and Synthesis of Novel Recombinant Genes Using PCR, PCR PRIMER A LABORATORY MANUAL, 603 (1994)] or QuikChangeTMSite-Directed Mutagenesis Kit (product made from STRATAGENE) using a primer with variation etc. can be used.

[0185] (2) Selection of an activity rise alteration variant [ as opposed to NF-kappa B activation according to the approach indicated to above-mentioned 2. ] is more possible than the variant of this polypeptide produced by selection (1) of the functional alteration variant of the polypeptide of this invention. The functional alteration variant which went up the NF-kappa B activation function can be obtained by introducing each of the variant of this polypeptide and this polypeptide into a reporter cell, and specifically choosing the variant which raised reporter activity from this polypeptide. Moreover, a dominant negative variant can be obtained by choosing the variant of this polypeptide that controls NF-kappa B activation under the stimulus existence which activates NF-kappa B.

[0186] The variant of this polypeptide is introduced into a reporter cell, and, specifically, it is cytokine (TNF-alpha), T cell mitogen, such as TNF-beta, IL-1alpha, IL-1beta, IL-2, and LIF (an antigen stimulus) Lectin, an anti-T cell receptor antibody, anti-CD2 antibody, anti-CD3 antibody, anti-CD28 antibody, calcium ionophore, and B cell mitogen (an anti-IgM antibody --) anti-CD40, leukotriene, LPS and PMA, a parasitism somesthesis stain, virus infection (it CMV(s) HIV-1, HTLV-1, and HBV and EBV --) HSV-1, HHV-6, NDV, Sendai Virus, adenovirus, etc., A virus product (double stranded RNA, Tax and HBX, EBNA-2, LMP-1 grade), DNA destructive matter and protein synthesis inhibitor (for example, cycloheximide) A dominant negative variant can be obtained by giving the stimulus which activates NF-kappa B, such as ultraviolet rays, a radiation, and oxidation stress, and choosing the variant of this polypeptide which fell rather than the time of reporter activity having not introduced the variant.

[0187] In addition, the obtained dominant negative variant (Dominant Negative mutants; dominant functional control variant) can be applied to inflammation response control or growth control of a malignant cell, and may be able to use for the gene therapy of the disease accompanied by activation of NF-kappa B DNA which carries out the code of this dominant negative variant. An example is raised to below and this invention is explained concretely. However, these examples are the things for explanation and do not restrict the technical range of this invention.

[0188]

[Example] From the [example 1] Homo sapiens large intestine, the large intestine of the \*production Homo sapiens of a Homo sapiens fat tissue origin perfect length cDNA library, and fat tissue, mRNA was extracted [ edition / 2nd / of molecular cloning ] by the approach of a

publication. Furthermore, polyA+RNA was refined by oligo dT cellulose. The cDNA library was produced from each polyA+RNA with Oligo-capping method [Gene, 138, and 171-174 (1994)]. According to the approach of a publication, composition of BAP (Bacterial A alkaline Phosphatase) processing, TAP (Tobacco Acid Phosphatase) processing, RNA ligation, and the first chain cDNA and removal of RNA were performed to a protein nucleic-acid enzyme, 41, 197-201 or (1996) Gene, 200, and 149-156 (1997) using Oligo-cap linker (array number 11) and Oligo dT primer (array number 12). The double strand cDNA was amplified by having used the first obtained chain cDNA as mold by PCR using two sorts of primers, the sense primer by the side of a five prime end (array number 13), and the antisense primer by the side of a three-dash terminal (array number 14), and it cut by SfiI. The commercial kit:GeneAmp XL PCR kit (product made from Perkin Elmer) was used, for 1 minute was repeated at 95 degrees C after heat treatment for 5 minutes, it repeated [ 95 degrees C ] the reaction cycle for 10 minutes 12 times for 1 minute and at 72 degrees C by 88 degrees C, and PCR performed it by holding at 4 degrees C after that.

[0189] The above-mentioned magnification cDNA was inserted in vector pME18SFL3 (GeneBank AB [009864 ], an expression vector, 3392bp) cut by DraIII, and the cDNA library was produced. About the plasmid DNA of each of the obtained clone, the base sequence of 5' edge and 3' edge of cDNA DNA sequencing reagent ( ) [ Dye Terminator ] Cycle SequencingFS Ready Reaction Kit and dRhodamine Terminator Cycle Sequencing FS Ready Reaction Kit or BigDye Terminator Cycle Sequencing FS Ready ReactionKit, and the product made from PE Biosystems are used. After performing a sequence reaction according to a manual, the base sequence was determined using the DNA sequencer (ABI PRISM 377, product made from PE Biosystems).

[0190] The artificial promoter who repeated the NF-kappa B recognition sequence in establishment IFN-beta of the reporter cell strain by which manifestation control of the luciferase activity is carried out by the [example 2] NF-kappa B enhancer (array number 15) 3 times was produced, and it inserted in 5' upstream region of the luciferase gene of a luciferase reporter vector (pAGE-luc; JP.3-22979A, the experimental medicine, 7, and 96-103 (1989)) (it is henceforth called pIF-luc). This plasmid 4microg was dissolved in TE buffer solution [10 mmol/l tris-HCl (pH8.0), 1 mmol/l EDTA (ethylenediaminetetraacetic acid sodium)] so that it might be set to 1micro g/mu.l, and transgenics was carried out to the Homo sapiens nephrocyte stock 293 (product made from Clontech) 1.6x106 piece by the electroporation method (the product made from BIO-RAD: Gene PulserTM). pIF-luc contains the hygromycin (hygromycin) resistance gene, and after transgenics established the stabilization transformant for culture and hygromycin as a selective marker of transgenics by the RPMI culture medium [RPMI1640 (Nippon Suisan Kaisha, Ltd. make), 10% calf blood serum, 0.05 mmol/l-mercaptoethanol, 25 U/ml penicillin G, and 25U/ml streptomycin] which added hygromycin 0.2 g/l. Among stabilization transformant, by TNF-alpha stimulus, the stock which guided the high luciferase activity of 670 times as compared with no stimulating was chosen (it is henceforth called 293-/IF-LUC), and it used for the following manifestation assays.

[0191] Shaking culture of the clone which determined the base sequence in the analysis example 1 over NF-kappa B activation of the perfect length DNA using [example 3] 293 / IF-LUC was respectively carried out at 37 degrees C for 16 hours among 2ml (Yeast ex tract 10 g/l, Trypton 16 g/l, NaCl 5 g/l) of 2xYT culture media which added ampicillin (100 mg/l). The centrifugal separator recovered the fungus body after culture, and the plasmid was respectively prepared by the approach of attachment data using the plasmid preparation kit (QIAPrep96 Turbo Miniprep Kit, product made from QIAGEN). It poured distributively so that it might become a plate with 20,000 per one well about 293 / IF-LUC cell 96 well, and it cultivated in the CO2 incubator at 37 degrees C for 16 hours. The RIPOFE cushion reagent (LIPOFECT AMINE 2000TM Reagent, product made from GIBCO BRL) was used for this cultured cell, respectively, and the 0.25micro of the above-mentioned plasmid abbreviation g was introduced into it according to the approach of attachment data. It used at 37 degrees C for 16 hours, a luciferase activity measurement reagent (LucLiteTM, product made from Packar) and luciferase activity measurement equipment (ARVO 1420 MULTILABEL COUNTER, product made from WALLC) were used after culture in the CO2 incubator, and luciferase activity was measured.

[0192] Consequently, COL03279 (DNA clone which has the base sequence of the array number 6), COL06772 (DNA clone which has the base sequence of the array number 7), ADKA01604 (DNA clone which has the base sequence of the array number 8), [when the plasmid of each clone of ADSU00701 (DNA clone which has the base sequence of the array number 9), and CAS01989 (DNA clone which has the base sequence of the array number 10) is introduced] As compared with negative control (pME18SFL3 is used), one 12.5 times, 6.3 times, 4.4 times, 2.7 times, and 3.0 times the activity of this was checked, respectively. DNA of this invention was respectively acquired from this clone.

[0193] the quantum of the amount of manifestations in the various organs of DNA of this invention accepted in each clone of the detection COL03279, COL06772, ADKA01604, and ADSU00701 of the amount of manifestations in the various organs of DNA of [example 4] this invention --- a law --- according to the method [PCR Protocols, Academic Press (1990), etc.], it carried out as follows using the half-quantitative PCR method. Moreover, the quantum of the transcript of the glyceraldehyde 3-phosphate dehydrogenase (glyceraldehyde-3-phosphate dehydrogenase:G3 PDH) considered to carry out the comparable manifestation in every cell was performed to coincidence, and it checked that it was practically equal to the conversion efficiency to a single strand cDNA from mRNA by the difference in the amount of mRNA(s) between cells, and the reverse transcriptase between samples.

[0194] mRNA of the Homo sapiens organ origin (the product made from Clontech: 3 caudate nucleus 2 brain 1 suprarenal gland) Four hippocampi, 5 substantia nigra, six thalami, the 7 kidney, the 8 pancreas, nine hypophyses, ten small intestines, Eleven bone marrow, 12 amygdalas, 13 cerebellums, 14 corpus callosa, 15 embryo brain, 16 embryo kidney, 17 embryo liver, 18 embryo lungs, the 19 heart, 20 liver, 21 lungs, 22 lymph gland, 23 mammary glands, 24 placentas, 25 prostate glands, 26 salivary glands, 27 skeletal muscle, 28 spines. The single strand cDNA was compounded using the cDNA composition kit (product made from SUPERScriptPTM Preamplification System: BRL) from 29 spleens, the 30 stomach, 31 testes, 32 thymus glands, the 33 thyroid, 34 tracheae, and 35 uteri. The single strand cDNA was compounded from mRNA of 1microg, and it diluted 240 times with water, and was used as mold of PCR. The synthetic DNA of a publication was used for the array numbers 16 and 17 based on the base sequence information from COL03279, the array numbers 18 and 19 based on the base sequence information from COL06772, the array numbers 20 and 21 based on the base sequence information from ADKA01604, and the array numbers 22 and 23 based on the base sequence information from ADSU00701 as a primer for PCR. The PCR reaction was performed according to the description using 10xGene Taq Universal Buffer and 2.5 mmol/LdNTP Mixture of NIPPON GENE Recombinant Taq DNA Polymerase (GeneTaq) and attachment. Thermal SAIKURA made from MJ RESERCH is used, and it is [degrees C / 94 ] 26 - 30 cycle \*\*\*\*\* about the reaction for 2 minutes for 1 minute and at 72 degrees C for 30 seconds and in 60 degrees C. Reaction mixture was analyzed by agarose gel electrophoresis and ethidium-bromide dyeing.

[0195] A result is shown in drawing 1 -4. DNA of this invention accepted in each clone of COL03279, COL06772, ADKA01604, and ADSU00701 had discovered the difference of strength by each clone and each organ by all 35 which a certain thing examined sorts of organs.

[0196]

[Effect of the Invention] According to this invention, allergy, atopy, asthma, pollinosis, respiratory tract irritation. The disease accompanied by activation of unusual immunocytes, such as an autoimmune disease and graft versus host disease, The endotoxin shock, septicemia, microorganism infection, chronic hepatitis B, chronic hepatitis C, An insulin dependency and non-dependency diabetes mellitus, glomerulonephritis, traumatic brain injury, psoriasis. The disease accompanied by infection and inflammation of gout, various encephalomyelitis, congestive heart failure, inflammatory bowel disease, etc., A Burkitt lymphoma, Hodgkin's disease, various lymphomas, adult T-cell leukemia, Unusual fibroblasts, such as a disease accompanied by unusual cell proliferations, such as a malignant tumor, rheumatoid arthritis, and hypertrophic arthritis, and the disease accompanied by activation of synovial membrane tissue, Viral diseases, such as an acquired immunodeficiency syndrome, the disease based on the failure of the nerve cell of ischemic encephalopathy, The disease based on the failure of nerve

cells, such as an Alzheimer disease and Parkinson's disease, The disease, multiple organ failure accompanied by unusual differentiation growth of smooth muscle cells, such as arteriosclerosis and restenosis, A systemic inflammatory response syndrome (SIRS:systemic inflammatory responsesyndrome), Retrieval of remedies, such as adult respiratory distress syndrome (ARDS:adult respiratorydistress syndrome), The antisense DNA/RNA of DNA and this DNA which carries out the code of a useful polypeptide and this polypeptide to development, The antibody which recognizes the gene therapy using this DNA and this polypeptide, the activity rise alteration object of this polypeptide, the dominant negative variants of this polypeptide, and these directions can be offered.

[0197]

[Array table free text]

Explanation of an array number 11-artificial array: Composition RNA (oligo cap linker array)

Explanation of an array number 12-artificial array: Synthetic DNA (oligo dT primer array)

Explanation of an array number 13-artificial array: Synthetic DNA (sense primer array by the side of a five prime end)

Explanation of an array number 14-artificial array: Synthetic DNA (antisense primer array by the side of a three-dash terminal)

Explanation of an array number 15-artificial array (transcription factor NF-kappa junction sequence)

Explanation of an array number 16-artificial array: Synthetic DNA (synthetic primer array which considered organization manifestation distribution)

explanation: of an array number 17-artificial array --- explanation: of a synthetic DNA array

number 18-artificial array --- explanation [ of a synthetic DNA array number 19-artificial array ]:

--- explanation [ of a synthetic DNA array number 20-artificial array ]: --- explanation [ of a synthetic DNA array number 21-artificial array ]: --- explanation [ of a synthetic DNA array number 22-artificial array ]: --- explanation [ of a synthetic DNA array number 23-artificial array ]: --- a synthetic DNA [0198]

[Layout Table]

SEQUENCE LISTING <110> KYOWA HAKKO KOGYO CO. and LTD. --- <120> Novel polypeptide<130> H12-0641J5<140 <141>> --- < --- 160> 21<170> PatentIn Ver2.1[0199 --- ] <210> 1<211> 780<212> PRT<213> Homo sapiens<400> 1Met Ala Ser Ala Glu Leu Glu-Gly-Lys-Tyr-Gln-Lys Leu Ala Glu 1 5 10 15 Tyr Ser Lys Leu Arg-Ala-Gln-Asn-Gln Val Lys Lys Gly-Val-Val 20 25 30 Asp Glu Gln Ala Asn Ser Ala Ala Leu Lys Glu Glu Lys Met Lys 35 40 45 Asp Gln Ser Leu Arg Lys Leu Gln Glu Met Asp Ser Leu Thr Phe 50 55 60 Arg Asn Leu Gln Leu Ala Lys Arg Val Glu Leu Gln Asp Glu 65 70 75 80 Ala Leu Ser GluPro Arg Gly Lys Lys Asn Lys Lys Ser Gly Glu Ser 85 90 95 Ser Ser Gln LeuSer Gln Glu Lys Ser Val Phe Asp Glu Asp Lys 100 105 110 Gln Lys Lys IleGlu Glu Asn Glu Arg Leu His Ile Gln Phe Phe Glu 115 120 125 AlaAsp Glu Gln HisLys His Val Glu Ala Glu Leu Arg Ser Arg Leu 130 135 140 Ala Thr Leu Glu ThrGlu Ala Ala GlnHis Gln Ala Val Val Asp Gly 145 150 155 160 Leu Thr Arg Lys TyrMet Glu Thr Ile Glu Lys Leu Gln Asn Asp Lys 165 170 175 Ala Lys Leu Glu Val Lys Ser Gln Thr Leu Glu Lys Glu Ala Lys Glu 180 185 190 Cys Arg Leu Arg Thr Glu Glu CysGlnLeu Gln Leu Thr Leu His 195 200 205 Glu Asp Leu Ser Gly Arg Leu Glu Ser Leu Ser Ile e Ile Asn Glu 210 215 220 Lys Val Pro Phe Asn Asp Thr Lys Tyr Ser Gln Tyr Asn Ala Leu Asn 225 230 235 240 Val Pro Leu His Asn Arg His Gln Leu Lys Met Arg Asp Ile Ala 245 250 255 Gly Gln Ala Leu Ala Phe ValGln Asp Leu Val Thr Ala Leu Asn 260 265270 Phe His Thr Tyr Thr Thr GlnArgLe Ile Phe Pro Val Asp Ser 275 280 285 Ala Ile Asp Thr Ile Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu 290 295 300 His Glu Asn Ala Ser Tyr Val Arg Pro Leu Glu Gly Met Lys His 305 310 315 320 Leu Phe Glu Ser Ile Thr Glu Asp Thr Val Thr Val Leu Glu Thr-Thr 325 330 335 Val Lys Leu Lys Thr Phe Ser Glu His-Leu-Thr-Ser-Tyr-Ile-Cys-Phe 340 345 350 Leu Arg Lys Ile Leu Pro Tyr Gln Leu Ser Leu Glu-Glu-Cys 355 360365 Glu Ser Ser Lys Cys Thr Ser Ala Leu Arg Ala Arg Asn Leu Leu 370 375 380 Ser Gln Asp Met Lys Lys Met Thr Thr Val Phe Glu Lys Leu Thr 385 390 395 400 Tyr Ile Ala Leu Leu Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu 405 410 415 Arg Thr Asn Tyr Ser Ser Val Leu Thr Asn Val Gly Ala Leu His 420 425 430 Gly Phe His Asp Val Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys 435 440 445 Ala Ala

Ile Glu His Glu Leu Pro Thr Ala Thr Thr Lys Lys Leu Thr 450 455 460 Thr Asn Asp Cys Ile Leu Ser Ser Val Val Ala Leu Thr Asn Gly Ala 485 470 475 480 Gly Lys Ile Ala Ser Phe Phe Ser Asn Asn Leu Asp Tyr Phe Ile Ala 485 490 495 Ser Leu Ser Tyr Gly Pro Lys Ala Ala Ser Gly Phe Ile Ser Pro Leu 500 505 510 Ser Ala Glu Cys Met Leu Glu Tyr Lys Lys Lys Ala Ala Tyr Met 515 520 525 Lys Ser Leu Arg Lys Pro Leu Leu Glu Ser Val Pro Tyr Glu Glu Ala 530 535 540 Leu Ala Asn Arg Arg Ile Leu Ser Thr Glu Ser Arg Glu Gly 545 550 555 560 Leu Ala Glu Glu Val Glu Glu Ser Leu Glu Lys Ile Ser Lys Leu Glu 565 570 575 Glu Glu Lys His Trp Met Leu Glu Ala Glu Leu Ala Lys Ile Lys 580 585 590 Leu Glu Lys Glu Asn Glu Arg Ile Ala Asp Lys Lys Asn Thr Gly 595 600 605 Ser Ala Glu Leu Val Gly Leu Ala Glu Glu Asn Ala Val Ser Asn 610 615 620 Thr Ala Gly Glu Asp Glu Thr Ala Lys Ala Val Leu Glu Pro Ile 625 630 635 640 Glu Ser Thr Ser Leu Ile Gly Thr Leu Arg Thr Ser Asp Ser Glu 645 650 655 Val Pro Asp Val Glu Ser Arg Glu Asp Leu Ile Lys Asn His Tyr Met 660 665 670 Ala Arg Ile Val Glu Thr Ser Glu-Leu-Leu-Ala-Asp-Ser-Lys 675 680 685 Ser Val His Phe Tyr Ala Glu Cys Arg-Ala-Leu-Ser-Lys-Arg-Leu-Ala 690 695 700 Leu Ala Glu Lys Ser Lys Glu Ala Leu Thr Glu Glu Met Lys Leu Ala 705 710 715 720 Ser Glu Asn Ile Ser Arg Leu Glu Asp Glu Thr Thr Thr Lys Arg 725 730 735 Ser Tyr Glu Asp Glu Ser Met Met Ser Asp His Leu Cys Ser Met 740 745 750 Asn Glu Thr Ser Lys Glu Arg Glu Glu Ile Asp Thr Leu Lys Met 755 760 765 Ser Ser Lys Gly Asn Ser Lys Lys Asn Lys Ser Arg 770 775 780 [0200]

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.\*\*\* shows the word which can not be translated.
3. In the drawings, any words are not translated.

## DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is the result of investigating the amount of manifestations of the COL03279 imprint object in 35 sorts of human tissues (organ) using \*\* and the PCR method.

[Drawing 2] It is the result of investigating the amount of manifestations of the COL06772 imprint object in 35 sorts of human tissues (organ) using \*\* and the PCR method.

[Drawing 3] It is the result of investigating the amount of manifestations of the ADKA01604 imprint object in 35 sorts of human tissues (organ) using \*\* and the PCR method.

[Drawing 4] It is the result of investigating the amount of manifestations of the ADSU00701 imprint object in 35 sorts of human tissues (organ) using \*\* and the PCR method.

[Description of Notations]

The figure of a publication in a complete diagram and the alphabet are as follows.

A suprarrenal gland, 02:brain, 03:caudate nucleus, 04:hippocampus, 05:substantia nigra, 06 : 01: A thalamus, 07 : The kidney, 08:pancreas, 09 hypophyses, 10:small intestine, 11:bone marrow, 12 : An amygdala, 13:cerebellum, 14:corpus callosum, 15:embryo brain, 16:embryo kidney, 17: Embryo liver, 18:embryo lungs, 19:heart, 20:liver, 21 : Lungs, 22: -- lymph gland and 23: -- a mammary gland, 24:placenta, 25:prostate gland, 26:salivary glands, 27:skeletal muscle, and 28: -- a spine, 29:spleen, 30:stomach, 31:testis, 32:thymus gland, and 33: -- the thyroid, 34:trachea, 35:uterus, Pr:plasmid, and M:molecular weight marker

[Translation done.]



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